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Canadian Environmental Protection Act, 1999

PRIORITY SUBSTANCES LIST

ASSESSMENT REPORT

CHLOROFORM

Environment Canada
Health Canada

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LIST OF ACRONYMS AND ABBREVIATIONS

ACH	air changes per hour
BMC	Benchmark Concentration
BMD	Benchmark Dose
CAS	Chemical Abstracts Service
CCPA	Canadian Chemical Producers' Association
CEPA	*Canadian Environmental Protection Act*
CEPA 1999	*Canadian Environmental Protection Act, 1999*
CFC	chlorofluorocarbon
CHO	Chinese hamster ovary
CI	confidence interval
CTV	Critical Toxicity Value
CYP	cytochrome P450
DBP	disinfection by-product
EC_{50}	median effective concentration
EEM	Environmental Effects Monitoring
EEV	Estimated Exposure Value
ENEV	Estimated No-Effects Value
EPA	Environmental Protection Agency (United States)
ETS	environmental tobacco smoke
FDA	Food and Drug Administration (United States)
FEP	fluorinated ethylene-propylene
GWP	Global Warming Potential
HCFC	hydrochlorofluorocarbon
ILSI	International Life Sciences Institute
IPCS	International Programme on Chemical Safety
K_{oc}	organic carbon/water partition coefficient
K_{ow}	octanol/water partition coefficient
kg-bw	kilogram body weight
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	Lowest-Observed-Adverse-Effect Level
LOEL	Lowest-Observed-Effect Level
NAPS	National Air Pollution Surveillance
NCI	National Cancer Institute (United States)
NOEC	No-Observed-Effect Concentration
ODP	Ozone Depletion Potential
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocyte
POCP	Photochemical Ozone Creation Potential
PSL	Priority Substances List
PTFE	polytetrafluoroethylene
RTECS	Registry of Toxic Effects of Chemical Substances

RWC	reasonable worst case
SCE	sister chromatid exchange
TC_{05}	Tumorigenic Concentration$_{05}$; concentration causing a 5% increase in tumour incidence over background
THM	trihalomethane
UDS	unscheduled DNA synthesis
VOC	volatile organic compound

Synopsis

Chloroform is used as a solvent and in the production of other chemicals. Its use as the feedstock for polytetrafluoroethylene and other fluoropolymers is increasing at approximately 4–6% per year. Significant releases of chloroform arise indirectly through reactions of chlorine with organic chemicals and as a by-product during the addition of chlorine to drinking water and wastewaters for disinfection. Although no manufacturing occurs in Canada, chloroform may still enter the environment through use and from deliberate and accidental release, as well as from long-range transport.

The majority of environmental releases of chloroform are to the atmosphere. In the troposphere, chloroform may be degraded by reaction with hydroxyl radicals or transported to the surface via washout. Chloroform released to surface waters or soil will be transported to the air because of its high volatility. It does not partition significantly from air to soils, sediments or tissues because of its low affinity for organic carbon and lipids. Chloroform may be quite persistent in groundwater because volatilization is limited, as there is no contact with air, and biodegradation is slow under anaerobic conditions and almost non-existent under most aerobic conditions. Chloroform does not appear to bioconcentrate in aquatic organisms to any significant extent, an observation that is consistent with its octanol/water partition coefficient (log K_{ow} = 1.97).

Chloroform has been detected in air, surface water, groundwater and drinking water in Canada.

Data on toxicity are available for microorganisms, algae, aquatic invertebrates, fish, amphibians and laboratory mammals. No data on toxicity were identified for birds or wild mammals. Chloroform in Canadian air, surface water and groundwater does not appear to pose significant risks to populations of terrestrial wildlife or aquatic biota.

Chloroform is not an effective agent of stratospheric ozone depletion, since net chlorine loading in the stratosphere from the substance itself and its degradation products is small. Chloroform does not contribute significantly to climate change or to ground-level ozone formation.

The general population in Canada is exposed to chloroform principally through inhalation of indoor air and ingestion of tap water. Estimated intake from a single daily 10-minute shower exceeds that from all other exposure pathways.

Chloroform has induced liver tumours in mice and renal tumours in mice and rats. The weight of evidence, which is strongest for hepatic and renal tumours in mice and more limited for renal tumours in rats, indicates that chloroform may be carcinogenic only at concentrations that induce the obligatory precursor lesions of cytotoxicity and proliferative regenerative response. This cytotoxicity is primarily related to rates of formation of reactive, oxidative metabolites, principally phosgene.

Non-cancer effects observed most consistently at lowest concentrations or doses following repeated exposures of rats and mice to chloroform are sustained cytotoxicity and persistent regenerative proliferation. As for cancer, target organs are the liver (centrilobular region) and kidney (cortical region). In addition, chloroform has induced nasal lesions in rats and mice exposed by both inhalation and ingestion at lowest concentrations or doses.

Modelled tissue dose measures for a 24-hour exposure scenario for the general population of Canada are less than those associated with values considered appropriate as Tolerable Concentrations/Doses for cancer and non-cancer effects. A Tolerable Concentration is the level to which it is believed a person may be exposed daily over a lifetime without deleterious effect.

Based on the information available, it is proposed that chloroform is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity; constitute or may constitute a danger to the environment on which life depends; or constitute or may constitute a danger in Canada to human life or health. Thus, it is proposed that chloroform not be considered "toxic" as defined in Section 64 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) and that evaluation of options under CEPA 1999 to reduce exposure not be considered a priority at this time. However, this is based on current use patterns; thus, future releases of this compound should continue to be monitored to ensure that exposure does not increase to any significant extent.

In view of the fact that showering is estimated to be the single greatest contributor to total daily intake of chloroform from drinking water, measures to reduce uptake from this source will be most effective in minimizing exposure of the general public.

1.0 INTRODUCTION

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) requires the federal Ministers of Environment and of Health to prepare and publish a Priority Substances List (PSL) that identifies substances, including chemicals, groups of chemicals, effluents and wastes, that should be given priority to determine whether they are harmful to the environment or constitute a danger to human health. The Act also requires both Ministers to assess these substances and determine whether they are "toxic" or are capable of becoming "toxic" as defined in Section 64 of the Act, which states:

> … a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that
> (a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
> (b) constitute or may constitute a danger to the environment on which life depends; or
> (c) constitute or may constitute a danger in Canada to human life or health.

Substances that are assessed as "toxic" as defined in Section 64 may be placed on Schedule I of the Act and considered for possible risk management measures, such as regulations, guidelines, pollution prevention plans or codes of practice to control any aspect of their life cycle, from the research and development stage through manufacture, use, storage, transport and ultimate disposal.

Based on an initial screening of readily accessible information, the rationale for assessing chloroform provided by the Ministers' Expert Advisory Panel on the Second Priority Substances List (Ministers' Expert Advisory Panel, 1995) was as follows:

> Chloroform is present in drinking water, food and indoor and outdoor air. Chloroform is imported for a variety of industrial applications and is generated in large quantities as a byproduct of water chlorination. Most environmental releases from industrial uses are to the atmosphere. Chloroform is carcinogenic and genotoxic in animals and there is some evidence that it is carcinogenic in humans. Information on this substance has been gathered, reviewed and evaluated by an international group of experts. An assessment is needed to characterize the level of exposure and the associated risks to human health and the environment in Canada.

Descriptions of the approaches to assessment of the effects of Priority Substances on the environment and human health are available in published companion documents. The document entitled "Environmental Assessments of Priority Substances under the *Canadian Environmental Protection Act*. Guidance Manual Version 1.0 — March 1997" (Environment Canada, 1997a) provides guidance for conducting environmental assessments of Priority Substances in Canada. This document may be purchased from:

Environmental Protection Publications
Environmental Technology Advancement Directorate
Environment Canada
Ottawa, Ontario

K1A 0H3

It is also available on the Internet at www.ec.gc.ca/cceb1/eng/psap.htm under the heading "Technical Guidance Manual." It should be noted that the approach outlined therein has evolved to incorporate recent developments in risk assessment methodology, which will be addressed in future releases of the guidance manual for environmental assessments of Priority Substances.

The approach to the assessment of effects on human health is outlined in the following publication of the Environmental Health Directorate of Health Canada: "*Canadian Environmental Protection Act* — Human Health Risk Assessment for Priority Substances" (Health Canada, 1994), copies of which are available from:

Environmental Health Centre
Room 104
Health Canada
Tunney's Pasture
Ottawa, Ontario
K1A 0L2

or on the Environmental Health Directorate publication web site (www.hc-sc.gc.ca/ehp/ehd/catalogue/bch.htm). The approach is also described in an article published in the *Journal of Environmental Science and Health — Environmental Carcinogenesis & Ecotoxicology Reviews* (Meek *et al.*, 1994). It should be noted that the approach outlined therein has evolved to incorporate recent developments in risk assessment methodology, which are described on the Environmental Substances Division web site (www.hc-sc.gc.ca/ehp/ehd/bch/env_contaminants/psap/psap.htm) and which will be addressed in future releases of the approach paper for the assessment of effects on human health.

The search strategies employed in the identification of data relevant to assessment of potential effects on the environment (prior to July 1999) and human health (prior to October 1999) are presented in Appendix A. Review articles were consulted where appropriate. However, all original studies that form the basis for determining whether chloroform is "toxic" under CEPA have been critically evaluated by staff of Environment Canada (entry and environmental exposure and effects) and Health Canada (human exposure and effects on human health).

The environmental sections of this report were produced by D. Moore and L. Pirie of the Cadmus Group, Inc. on behalf of Environment Canada and were revised by D. Caldbick and K. Taylor, Environment Canada. They were reviewed by the following members of the Environmental Resource Group, established by Environment Canada to support the environmental assessment:

P. Doyle, Environment Canada
W. Hayes, Dow Chemical
K. Kaiser, Environment Canada

Environmental sections of the Assessment Report and the environmental supporting documentation (Environment Canada, 1999a) were also reviewed by internal reviewers at Environment Canada — namely, P. Cureton and D. Dubé — as well as by external reviewers:

D. Averill, Water Technology International Corporation
W.J. Birge, University of Kentucky
N. Bunce, University of Guelph
D.B. Carlisle, Brez-Carlisle Inc.
L. Gammie, AQUALTA
D. Gessford, Dow Chemical Company
M.D. Kercher, University of Kentucky
D.J. Price, University of Kentucky

The health-related sections of this Assessment Report are based in part on the deliberations of two expert groups, in which staff of Health Canada participated. These were a Task Group on chloroform of the International Programme on Chemical Safety (IPCS) (WHO, 1994) and an expert panel convened by the International Life Sciences Institute (ILSI) to develop case studies for chloroform and dichloroacetic acid in the context of the revised cancer guidelines released in 1996 by the U.S. Environmental Protection Agency (EPA) (ILSI, 1997)

The IPCS monograph on the health effects of chloroform was published in 1994. The first draft was prepared by Dr. J. de Fouw of the National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands, and subsequently circulated to IPCS focal points for comment. The revised draft was subsequently finalized at a meeting of the following Task Group members, held in Geneva on November 15–19, 1993:

M.W. Anders, University of Rochester, Rochester, New York, USA
D. Anderson, British Industrial Biological Research Association, Surrey, United Kingdom
R.J. Bull, Washington State University, Pullman, Washington, USA
C.D. Carrington, Food and Drug Administration (FDA), Washington, D.C., USA
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J. de Fouw, National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands (Rapporteur)
E. Elovaara, Institute of Occupational Health, Helsinki, Finland
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The ILSI expert panel, which first met in September 1996, was composed of the following members:

M. Andersen, ICF Kaiser International (Chair)
G. Boorman, National Institute of Environmental Health Sciences
D. Brusick, Covance Laboratories, Inc.
S. Cohen, University of Nebraska Medical Center
Y. Dragan, McArdle Laboratory for Cancer Research
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J. Goodman, Michigan State University
G. Hard, American Health Foundation
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E. O'Flaherty, University of Cincinnati

The final draft of the expert panel report on chloroform was reviewed externally by:

C. Klaassen, University of Kansas Medical Center
R. Melnick, National Institute of Environmental Health Sciences
L. Rhomberg, Harvard Center for Risk Analysis.

The outcome of these assessments has been updated and considered in the context of the approach to assessment of "toxic" under Paragraph 64(c) of CEPA 1999. In addition, the physiologically based pharmacokinetic (PBPK) model for animals included in ILSI (1997) was refined and a human component developed by the K.S. Crump Group (ICF Kaiser, 1999).

The content of the health-related sections of this Assessment Report and the supporting documentation (Health Canada, 1999) were prepared by the following staff of Health Canada:

R. Beauchamp
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The section related to genotoxicity was reviewed by D. Blakey, Environmental and Occupational Toxicology Division. The PBPK model incorporated herein was reviewed externally by M. Gargas, ChemRisk, McLaren Hart Inc.

The health-related sections on toxicity in the Assessment Report were reviewed externally by:

M. Andersen, Colorado State University
B. Butterworth, Chemical Industry Institute of Toxicology
J. Wiltse, Office of Water, U.S. EPA
D. Wolf, National Health and Environmental Effects Research Laboratory, U.S. EPA

The health-related sections of the Assessment Report were reviewed and approved by the Health Protection Branch Risk Management meeting of Health Canada.

The entire Assessment Report was reviewed and approved by the Environment Canada/Health Canada CEPA Management Committee.

The text of the Assessment Report has been structured to address environmental effects initially (relevant to determination of "toxic" under Paragraphs 64(a) and (b)), followed by effects on human health (relevant to determination of "toxic" under Paragraph 64(c)).

Copies of this Assessment Report are available upon request from:

Inquiry Centre
Environment Canada
Main Floor, Place Vincent Massey
351 St. Joseph Blvd.
Hull, Quebec
K1A 0H3

or on the Internet at:

www.ec.gc.ca/cceb1/eng/public/index_e.html

Unpublished supporting documentation (Environment Canada, 1999a; Health Canada, 1999), which presents additional information, is available upon request from:

Commercial Chemicals Evaluation Branch
Environment Canada
14th Floor, Place Vincent Massey
351 St. Joseph Blvd.
Hull, Quebec
K1A 0H3

or

Environmental Health Centre
Room 104
Health Canada
Tunney's Pasture
Ottawa, Ontario
K1A 0L2

2.0 SUMMARY OF INFORMATION CRITICAL TO ASSESSMENT OF "TOXIC" UNDER CEPA 1999

2.1 Identity and physical/chemical properties

The Chemical Abstracts Service (CAS) registry number for chloroform is 67-66-3, and the Registry of Toxic Effects of Chemical Substances (RTECS) registry number is FS 9100000. Common synonyms include trichloromethane, methane trichloride, trichloroform, methyl trichloride, methenyl trichloride, formyl trichloride, chloroformum and chloroformium anesthesicum. Registered trade names include Freon 20, R 20 and R 20 refrigerant (WHO, 1994).

Chemical and physical properties of chloroform are presented in Table 1. Chloroform is a clear, colourless and volatile liquid with a burning sweet taste and pleasant, etheric odour. Pure chloroform is light sensitive; thus, reagent chloroform is usually stabilized with 0.75% ethanol or 0.006% amylenes to avoid photochemically induced oxidations to phosgene and hydrogen chloride (WHO, 1994). Chloroform is miscible in many organic solvents and is moderately soluble in water.

2.2 Entry characterization

2.2.1 Production, importation and uses

Chloroform has not been manufactured in Canada since 1978.

Canadian imports of chloroform were 402 tonnes in 1993, 69 tonnes in 1995 and 118 tonnes in 1996, most of which came from the United States (SRI, 1995; Environment Canada, 1997b). Imports have declined in recent years because the major importer, Allied-Signal Canada Inc., closed its HCFC-22 (a hydrochlorofluorocarbon) plant in Amherstburg, Ontario, in 1992.

Chloroform is used in the production of other chemicals and as a solvent. It is used as a precursor in the production of HCFC-22. The quantities of HCFC-22 used in refrigerant applications are decreasing because of the Copenhagen Amendment of the Montreal Protocol (which called for a phaseout in HCFC production), but its use as the feedstock for polytetrafluoroethylene (PTFE), fluorinated ethylene-propylene (FEP) resin and other fluoropolymers is increasing at approximately 4–6% per year (Gessford, 1997). The net effect is that the chloroform demand for HCFC-22 production is fairly constant. Prior to and during World War II, chloroform was used primarily as an anesthetic and in pharmaceutical preparations (National Research Council, 1978). Its use as an anesthetic has been largely discontinued, although it has limited medical use in some dental procedures and in the administration of drugs for the treatment of some diseases (ATSDR, 1995). Chloroform has been used in the past as an extraction solvent for fats, oils, greases, resins, lacquers, rubber, alkaloids, gums, waxes, gutta-percha, vitamins, flavours, floor polishes, penicillin and other antibiotics, as an adhesive in artificial silk manufacture and as an intermediate in the preparation of dyes and drugs (ATSDR, 1995). The U.S. FDA banned the use of chloroform as an ingredient in human drugs, cosmetics and food packaging products in 1976 (Windholz, 1983).

The use of chloroform in cosmetics is not permitted in Canada under Section 15 of the Cosmetics Regulations of the *Food and Drugs Act* (Denman, 1999). Chloroform is neither an active product nor a formulant in any registered pesticide product in Canada (Moore, 1999). Under the Consumer Chemicals and Containers Regulations of the *Hazardous Products Act*, labelling is required for polishes, cleaning agents, liquid coating materials and paint and varnish removers containing chloroform at concentrations greater than 0.4% and more than 100 mg (Chowhan, 1999). Under the *Food and Drugs Act*, manufacturers are not permitted to import or sell a drug that contains chloroform for human use in Canada (Pon, 1999).

World supply/demand is expected to stay fairly constant over the next decade, as the anticipated decrease in production of refrigerants should be offset by the increased use of HCFC-22 as a fluoropolymer feedstock. HCFC-22 will be phased out between 2010 and 2020, effectively eliminating most of the present market for chloroform.

2.2.2 Sources and releases

Although chloroform is no longer manufactured in Canada, it can enter the environment through deliberate and accidental releases. It can also enter the Canadian environment via long-range atmospheric transport of releases from facilities in other countries. Chloroform is formed as a by-product during the addition of chlorine to drinking water and wastewater for disinfection (ATSDR, 1995). Other significant releases of chloroform arise indirectly through reactions of chlorine with organic chemicals. Major sources and releases of chloroform are discussed in more detail below. Minor sources include exhaust emissions from vehicles as a result of the decomposition of 1,2-dichloroethane added to fuel as a lead scavenger, decomposition of trichloroethylene and 1,1,1-trichloroethane in the atmosphere, and paper bleaching with chlorine (WHO, 1994).

2.2.2.1 Natural sources

A variety of organic compounds present in natural waters may contribute to the formation of trihalomethanes (THMs), including chloroform, but only if the water is chlorinated. The major group of precursors are the humic and fulvic acids derived from soils and the decomposition of plant material. Nightingale *et al.* (1995) observed the natural production of chloroform by marine macroalgae both *in situ* in the North Sea and in the laboratory. The quantities of chloroform formed from natural sources are unknown.

2.2.2.2 Anthropogenic sources

Anthropogenic sources of chloroform include pulp and paper mills, municipal wastewater treatment plants, chemical manufacturing plants and waste incinerators.

In an industrial survey conducted under CEPA Section 16, 23 pulp and paper mills reported releases of 288 tonnes of chloroform into the atmosphere, 15.6 tonnes into water bodies, 0.019 tonnes into wastewater treatment plants and 0.127 tonnes into landfills in 1996 (Environment Canada, 1997b). In the pulp bleaching process, 80–90% of the chloroform production occurs during the hypochlorite stage, and additional amounts are formed during chlorination and extraction, with only traces produced during the chlorine dioxide stage (Hrutfiord and Negri, 1990). The production of chloroform is generally correlated with the lignin content of the pulp and with the amount of chlorine applied. The formation of chloroform is reduced by substituting chlorine dioxide for elemental chlorine in the pulp bleaching process (Crawford and Stryker, 1988; Crawford *et al.*, 1991). In the 1980s, the need to reduce chlorinated by-products in the effluent streams of pulp and paper mills using elemental chlorine to bleach pulp was identified (Environment Canada, 1992). In particular, Regulations under CEPA were promulgated respecting the release of polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans in effluents from pulp and paper mills. These regulations were intended to ensure that the pulp and paper mills implement process changes preventing the formation of these and other chlorinated by-products. Chloroform was one of the many chlorinated by-products that were reduced in the effluents of these mills as a consequence of the Regulations. Environmental Effects Monitoring (EEM) provisions were implemented to determine the adequacy of the effluent regulations. As a result of the measures taken to prevent their formation, the total discharge of all dioxins and furans has fallen by approximately 99%, from about 450 g in 1988 to about 5 g per year at the end of 1994 (Environment Canada, 1996a). Recently, a large pulp and paper plant in northern Alberta reported a 98% reduction in chloroform concentrations measured in their wastewater following the replacement of elemental chlorine with chlorine dioxide in their bleach plant. In 1997, a chloroform concentration of 104 µg/L was measured in the effluent from the plant while it was using elemental chlorine to bleach the pulp, and this was reduced to 2 µg/L following the changeover to chlorine dioxide in 1998 (Henteleff, 1999). Chlorine dioxide itself does not tend to form THMs such as chloroform. However, chlorine impurities in chlorine dioxide feed or the use of secondary disinfection with chlorine following primary disinfection with chlorine dioxide can lead to THM formation (Dahlman *et al.*, 1993). According to Solomon *et al.* (1994), levels of chloroform generated in the bleach plant drop 1000-fold from about 470 g/tonne at 10% substitution of chlorine dioxide for elemental chlorine (Stinchfield and Woods, 1994) to 0.4 g/tonne at 100% substitution (Wiesemann, 1994). It had been predicted earlier that Canadian production of chemically bleached pulp using chlorine dioxide would grow by 7% to 8.7 million tonnes in 1997, accounting for more than 76% of the market (AET, 1998).

Total on-site environmental releases of chloroform reported to the National Pollutant Release Inventory in 1996 were 208 tonnes (NPRI, 1999). Almost all of this was released from the pulp, paper and allied products industry. More than 96% of these releases were to the atmosphere, with the remainder being released to water.

Although releases to the Canadian environment have not been quantified, municipal wastewater treatment plant disinfection systems that use chlorine can be significant sources of chloroform (Robinson and Desing, 1995). Of the four most common THMs in wastewaters (chloroform, dibromochloromethane, dichlorobromomethane and bromoform), chloroform is the volatile organic usually present in the highest concentration. Chloroform and other THMs are produced by the reaction of chlorine with organic precursor molecules in the "haloform reaction." The major precursors for the formation of chloroform are humic and fulvic acids derived from soils and the decomposition of plant material (Itoh *et al.*, 1985; Wardlaw *et al.*, 1991). Environment Canada (1999a) provides more information about this chemical reaction. The quantities of chloroform released to the Canadian environment from wastewater treatment plants that chlorinate for disinfection are not known. Chloroform releases are highly variable, depending on the flow rate handled at the treatment plants and on the chemical conditions at the plants.

Chloroform can also be released from industrial plants. The CEPA Section 16 survey revealed that three of the Canadian Chemical Producers' Association (CCPA) member company facilities released a total of 145 kg chloroform in 1996, of which 88% was released to air (Environment Canada, 1997b). Emission factors for chloroform consumed in the production of HCFC-22 range from 0.077 to 2.58 kg chloroform per tonne HCFC-22 (WHO, 1994). Using these factors, chloroform releases due to HCFC-22 production in Canada were estimated to have ranged from 31 to 1040 kg in 1993.The CCPA estimated that its member companies released 540 kg chloroform to the Canadian environment in 1992 (CCPA, 1992).

Other sources of chloroform releases to air and water include hazardous waste incinerators (Jay and Stieglitz, 1995; Hart *et al.*, 1996), medical waste incinerators (mean emission factor, 8.1–119 µg/kg waste; mean annual emissions to air for 14 plants in Alberta in 1987, ~3.4 kg) (Walker and Cooper, 1992), waste composting facilities (Eitzer, 1994), residential washing machines (1200 mg per person per year, most of which is discharged to treatment plants) (Shepherd *et al.*, 1996) and various household products and indoor materials (Ozkaynak *et al.*, 1987). These sources would be minor compared with pulp and paper mills and wastewater treatment plants.

2.3 Exposure characterization

2.3.1 Environmental fate

2.3.1.1 Air

The majority of environmental releases of chloroform are to the atmosphere, where chloroform exists almost entirely in the vapour phase (Eisenreich *et al.*, 1981).

Reaction with photochemically generated hydroxyl radicals is the most important mechanism for decomposition of chloroform in the troposphere (Kindler *et al.*, 1995). The rate constant for this reaction at 25°C has been experimentally determined in several studies and ranges from 1.0×10^{-13} to 2.95×10^{-13} cm^3 per molecule per second (Derwent and Eggleton, 1978; Singh *et al.*, 1981; Klöpffer *et al.*, 1988). The higher rate was determined under photochemical smog conditions (Klöpffer *et al.*, 1988). Based on a 12-hour sunlit day in a typical atmosphere at warmer latitudes containing 1×10^6 hydroxyl radicals/cm^3, the average residence time for chloroform in the troposphere would be 116 days (Singh *et al.*, 1981). Depending on the rate constant chosen for reaction of hydroxyl radicals with chloroform, temperature, hydroxyl radical concentration, latitude and other factors, chloroform half-lives in the troposphere could vary from 54.5 days (Klöpffer *et al.*, 1988) to 620 days (Singh *et al.*, 1978). Shorter half-lives are expected under warm, photochemical smog conditions, while much longer half-lives would be expected at latitudes with colder temperatures and shorter sunlit days. Assuming an atmospheric half-life of 193 days, Kindler *et al.* (1995) predicted that 1.7% of the chloroform in the troposphere will migrate to the stratosphere. The chloroform half-life in the stratosphere was estimated to be 3.18 years (Kindler *et al.*, 1995).

Phosgene, the main metabolite responsible for chloroform toxicity, is an important product of the photooxidation reaction of chloroform. Other reaction products include dichloromethane, formyl chloride, carbon monoxide, carbon dioxide and hydrogen chloride (Gürtler and Kleinermanns, 1994).

Direct photolysis in the atmosphere is not a significant degradation process because chloroform does not absorb light significantly at wavelengths above 250 nm (Crookes *et al.*, 1994).

Due to its solubility, some chloroform should be removed from the atmosphere via wet deposition. Edwards *et al.* (1982) and Atkinson (1990) quantified this process, however, and found it to be minor. Of the chloroform removed by deposition, most will return to the atmosphere by volatilization from surface waters and soil (Diamond *et al.*, 1994).

Since chloroform has a half-life in the atmosphere of up to 620 days, long-range transport to remote areas and to the stratosphere is possible (Mackay *et al.*, 1993).

2.3.1.2 Water

The principal fate process for chloroform in surface waters not covered by ice is volatilization. In modelling studies, the half-life for volatilization has been estimated to range from 36 hours in a river to 9–10 days in a lake (U.S. EPA, 1984). Shorter half-lives have been estimated in other modelling studies, particularly for shallow, well-mixed systems with high wind velocities (e.g., Kaczmar, 1979; Lyman *et al.*, 1982). Large releases can lead to bottom accumulations because the liquid density of chloroform is higher than that of water (Pecher and Herrmann, 1986). This phenomenon was observed by Neely *et al.* (1976) following a spill of 800 tonnes of chloroform into the Mississippi River.

There are conflicting data on the biodegradation of chloroform in aquatic systems. Under aerobic conditions, there has been little biodegradation after up to 25 weeks in most studies (e.g., Bouwer *et al.*, 1981; Wilson *et al.*, 1981, 1983; Bouwer and McCarty, 1984), although significant losses have been claimed in a few studies (e.g., Flathman and Dahlgran, 1981; Tabak *et al.*, 1981). These latter studies, however, did not eliminate the possibilities of volatilization, adsorption to test apparatus or formation of micro-anaerobic zones. Under specialized conditions (e.g., presence of methanogenic or nitrifying bacterial populations), chloroform can be co-metabolized with an energy source (e.g., methane, ammonia) and mineralized to carbon dioxide and chlorides (Bouwer and McCarty, 1983; Oldenhuis *et al.*, 1991). The relevance of this aerobic biodegradation pathway in the natural environment is limited.

In surface water, chemical degradation rates via hydrolysis, photolysis or reaction with photochemically produced hydrated electrons are probably too slow to compete with volatilization as a removal process (Mabey and Mill, 1978; Mill *et al.*, 1980; Jeffers *et al.*, 1989).

2.3.1.3 Sediment and soil

Chloroform is not expected to partition significantly to soils, bottom sediments or suspended organic matter in surface water because of its low affinity for organic carbon and lipids. The EXAMs model used by Anderson *et al.* (1985) predicted that the percentage of total chloroform in water transferred to bottom sediments would be 3% for a river, 8% for a pond and <0.06% for a lake.

At the soil surface, the principal fate of chloroform is volatilization, because of its high volatility and low soil adsorption. In a microcosm study in which 2.7 mg of chloroform were added each day for 12 weeks, Piwoni *et al.* (1986) estimated that 75% (95% confidence interval [CI] = ±19%) of the chloroform in wastewaters applied to land volatilized to the atmosphere, while the remainder leached through the microcosm soil. In a similar study, in which chloroform was added in a feed solution at a concentration of 74.1 µg/L and 146.6 µg/L 1 day per week for 10 weeks, 37% volatilized at 12°C and 39% at 21°C (Park *et al.*, 1988). Soil type and concentrations of chloroform did not affect volatilization, but volatilization increased with temperature. Grathwohl (1990), Walton *et al.* (1989, 1992) and others have measured the organic carbon/water partition coefficients (K_{oc}) in a wide variety of soils and sediments and reported that the K_{oc} is generally low, ranging from 27.5 for sandy soil to 617 for unweathered shale and mudrock. Dural and Peng (1995) demonstrated that what soil sorption does occur is the result of adsorption (soil–solute interactions). As a result, chloroform sorption is correlated with soil clay content.

Based on data on degradation in water and limited studies in soil and sediment, chemical degradation of chloroform is not expected to be rapid in soil and sediment, except under anaerobic methanogenic conditions. The major products of anaerobic degradation in soil and sediment are carbon dioxide, methane and hydrogen chloride. Small amounts of dichloromethane are also produced. Under anaerobic conditions, Van Beelen and Van Keulen (1990) reported that chloroform had a half-life of 12 days at 10°C and 2.6 days at 20°C. Van Beelen and Van Vlaardingen (1993) observed that chloroform had a half-life of 2–37 days in anaerobic, muddy sediments (2.5–8.7% organic carbon content). In contrast, anaerobic, sandy sediments (0.2–0.3% organic carbon content) did not show mineralization of chloroform. Based on these results, the authors speculated that the inactivity of chloroform-mineralizing bacteria in sandy sediments might allow chloroform from infiltrating river water to reach groundwater.

The generally low organic content of subsoils and poor sorption of chloroform to soils with low organic content indicate that this substance has the potential to leach to groundwater (Wilson *et al.*, 1981; Piwoni *et al.*, 1986). In groundwater, chloroform may be quite persistent, because volatilization is limited, and biodegradation is slow under anaerobic conditions or non-existent under most aerobic conditions. Slow reductive dehalogenation of chloroform under anaerobic conditions to produce dichloromethane and chloromethane has been demonstrated in a large number of studies (e.g., Bouwer *et al.*, 1981; Gossett, 1985; Gälli and McCarty, 1989; Krone *et al.*, 1989; Hughes, 1992; Rhee and Speece, 1992; Long *et al.*, 1993). Since most of these studies were conducted in chemostats or other specialized bioreactors, it is difficult to determine what reaction rates one might expect in the natural environment. *In situ* anaerobic biodegradation has been observed in contaminated groundwaters at the DuPont industrial landfill in Niagara Falls, New York. At this site, high levels of nitrogen and biodegradable compounds were present to support co-metabolic microbial activity.

2.3.1.4 Biota

The octanol/water partition coefficient of chloroform (log K_{ow} = 1.97) indicates that the substance is unlikely to bioaccumulate to any significant extent in aquatic biota (Anderson and Lusty, 1980). Laboratory studies have indicated bioconcentration factors of 690 in green algae (Mailhot, 1987), 2–6 in bluegill (*Lepomis macrochirus*) (Veith *et al.*, 1978; Anderson and Lusty, 1980; Barrows *et al.*, 1980), 5–10 in rainbow trout (*Oncorhynchus mykiss*) (Anderson and Lusty, 1980), 1.4–2.2 in largemouth bass (*Micropterus salmoides*) and 3–3.4 in channel catfish (*Ictalurus punctatus*). No data regarding potential for biomagnification are available; however, in view of its low K_{ow} and rapid depuration rates in aquatic biota (a half-life of less than 1 day in rainbow trout, bluegills, largemouth bass and channel catfish) (Anderson and Lusty, 1980; Barrows *et al.*, 1980), significant biomagnification of chloroform is unlikely.

Darnerud *et al.* (1989) studied the distribution of radiolabelled chloroform in rainbow trout. During the exposure period, they observed the highest levels of radioactivity in bile and decreasing amounts in liver, intestinal mucosa, kidney, brain, gills and olfactory rosette. Under clean conditions, the level of radioactivity decreased, with the highest levels observed in the intestinal mucosa, liver and central nervous system. The major part of this radioactivity was thought to be tissue-bound metabolites.

2.3.2 Environmental concentrations

In the Canadian environment, chloroform has been frequently detected in air and water. Information on concentrations in food is more limited, and few studies on levels in sediment, soil and biota have been identified. Data presented here are restricted to those collected in Canada, except where such information was not identified or was extremely limited. Concentrations of chloroform in air in the United States are also reported due to potential for long-range transport in this medium.

2.3.2.1 Ambient air

Chloroform was detected (i.e., at greater than 0.1 μg/m^3) in more than 69% of 8807 24-hour samples collected from 47 sites in seven provinces between 1989 and 1996 (Dann, 1998). During this period, annual median concentrations ranged from <0.1 to 0.18 μg/m^3, and annual arithmetic mean concentrations ranged from 0.12 to 0.23 μg/m^3. Concentrations in ambient air were lowest at rural sites, higher in suburban and urban areas and highest at National Air Pollution Surveillance (NAPS) sites immediately adjacent to major roadways in urban areas (Dann, 1998). Comparison of the distributions of concentrations for the periods 1989–1992 (n = 3344) and 1993–1996 (n = 5463) indicates that the concentrations of chloroform in ambient air in Canada in the more recent period were slightly less than previous values (Health Canada, 1999). The highest 24-hour average concentration measured between 1989 and 1996 was 6.0 μg/m^3 at a suburban site near Vancouver, British Columbia, in 1992. The highest concentration measured during 1996 was 0.75 μg/m^3.

Outside Canada, chloroform levels in urban and source-dominated areas in the United States were 0.3–9.9 μg/m^3 and 4.1–110 μg/m^3, respectively (ATSDR, 1996).

2.3.2.2 Indoor air

Twenty-four-hour indoor air samples were collected using passive sampling devices from 754 residences in nine provinces during a national survey in Canada from January 1991 to January 1992 (Concord Environmental Corporation, 1992). The residences were selected from census subdivisions to be representative of all regions of Canada. Samples were collected from living areas (i.e., living room, dining room or family room), and bathrooms and kitchens were intentionally avoided. As a consequence of a relatively high limit of detection (i.e., 3.5 μg/m^3), chloroform was detected in only 11% of the 754 samples, at a maximum concentration of 68.6 μg/m^3. The arithmetic mean concentration, which was less than the limit of detection, was estimated as follows. The distribution of concentrations of chloroform in these indoor air samples was assumed to be lognormal in shape, and a method involving least squares estimation was used to impute values below the detection limit, resulting in an estimated mean concentration of 2.3 μg/m^3 (Health Canada, 1999).

Passive sampling devices were also used to collect 24-hour air samples during a Health Canada-sponsored multimedia exposure study in 1996 and 1997. Initially, a pilot study was conducted in the Greater Toronto Area involving 44 randomly selected households (Otson and Meek, 1996). At a limit of detection of 2.3 μg/m^3, chloroform was detected in only 8 of 44 samples of indoor air, at a maximum concentration of 14.1 μg/m^3. The second phase of the study involved 50 randomly selected households in the Greater Toronto Area, Nova Scotia and Alberta (Conor Pacific Environmental, 1998). The limit of detection for the 24-hour air samples was 0.22 μg/m^3. Chloroform was detected in 34 of the 50 samples, at a maximum concentration of 7.1 μg/m^3. The overall (censored) mean concentration for the 94 samples of indoor air was 1.5 μg/m^3, when a concentration equivalent to one-half the appropriate limit of detection was assumed for each sample in which chloroform was not detected (Health Canada, 1999). A 24-hour personal air sample was also collected from the breathing zone of a single participant at each of the 94 households.

Concentrations of chloroform ranged from <0.22 to 94.5 μg/m^3 in these personal air samples, with an overall (censored) mean concentration of 2.6 μg/m^3.

A detailed questionnaire was administered to each participant to obtain information on the characteristics of the residence and the activities of the participant and other members of the household (Otson and Meek, 1996). Analysis of the responses did not reveal any potential sources of chloroform in the indoor air of these households. In particular, there were no statistically significant correlations between the 24-hour average concentrations of chloroform in the indoor air of the residences and the durations of tap water usage (e.g., for showering or bathing, washing clothes, or washing dishes) on the day sampled (Health Canada, 1999).

Limits of detection varied with sample site categories and sampling durations (Bell *et al.*, 1993). Chloroform was detected in 89 (or 61%) of 146 indoor air samples in Windsor, Ontario, collected by active sampling (i.e., portable sampling pump and sorbent tube) in the Windsor Air Quality Study conducted during 1991 and 1992 (OMEE, 1994). The frequencies of detection were highest (i.e., 87–100%) at sites where environmental tobacco smoke (ETS) was present. The highest mean concentration of chloroform in the indoor air of "non-smoking" locations was 5.6 μg/m^3, while mean concentrations ranged up to 16 μg/m^3 at the sites where ETS was present.

In the United States, mean concentrations of chloroform in the indoor air of residences ranged from 0.17 to 43.9 μg/m^3, with a maximum reported value of 210 μg/m^3 (Samfield, 1992). The mean concentration in the indoor air of 61 "non-smoking" homes in Mt. Laurel, New Jersey, in 1992 was 0.60 μg/m^3 (Heavner *et al.*, 1996). The mean concentration was 0.85 μg/m^3 in 32 homes where smoking was permitted. Twelve-hour samples of indoor air ($n = 248$) were collected in the kitchens and living rooms of homes in Los Angeles, California, in 1987 (Wallace, 1997). Mean concentrations ranged from 0.9 to 1.5 μg/m^3, and the maximum concentration was 13 μg/m^3.

Concentrations of chloroform in indoor air may be elevated for short periods of time due to volatilization during use of heated tap water . Showers are particularly effective in releasing dissolved volatile organic compounds (VOCs), including chloroform, to indoor air (Andelman, 1985), and more than 50% of the chloroform in the warm water entering through a shower head may be volatilized into the air in a shower compartment (Tancrède *et al.*, 1992; Giardino and Andelman, 1996). During showering, the concentration of chloroform in the air of the shower compartment increases rapidly and may exceed 1000 μg/m^3 when there is minimum air exchange between the shower compartment and the adjacent (e.g., bathroom) area (Health Canada, 1999). Concentrations are highest in the breathing zone of a showering individual, due to the proximity of the shower head (Benoit *et al.*, 1997). Similarly, short-term elevated concentrations of chloroform in air result during bathing and hot tub use, while longer-term elevated concentrations can occur in buildings with indoor swimming pools (Health Canada, 1999).

2.3.2.3 Surface water

High concentrations of chloroform have occasionally been reported for Canadian surface waters, with the highest concentrations occurring in surface waters near pulp and paper mills using chlorine bleaching. For example, concentrations of chloroform below the Canadian Pacific Forest Products Kraft Mill in Thunder Bay, Ontario, ranged from 80 to 200 µg/L in 1986 (OMOE, 1990). In 1989, levels in the Fraser River near Prince George, British Columbia, below the Northwood Pulp and Timber outfall reached 83 µg/L, with a mean concentration of 27 µg/L and a median concentration (n = 8) of 2.5 µg/L (B.C. MOE, 1989).

While no data exist with which to directly compare pre- and post-1990 levels at Thunder Bay or Prince George, in more recent studies, i.e., since 1990, significantly lower concentrations of chloroform in Canadian surface waters have been reported. Concentrations of chloroform ranging up to 7 µg/L were reported for sources of drinking water (surface or well water) from Alberta in 1990–1995. Only 2 (5 µg/L and 7 µg/L) of 59 samples contained concentrations of chloroform above the 1 µg/L detection limit (Alberta Environment, 1996). Almost all surface water samples reported on the NAQUADAT database (n = 321, 1990–1996, all from Alberta) contained concentrations of chloroform below the 1 µg/L detection limit. The highest concentration reported was 2 µg/L (Environment Canada, 1996b). Concentrations of chloroform in surface water from British Columbia ranged up to 18 µg/L in 1990–1995. Two (5.9 µg/L and 18 µg/L) of 12 samples contained concentrations of chloroform above the 1 µg/L detection limit (B.C. MOE, 1996). Chloroform concentrations ranging from <0.001 to 4.2 µg/L were reported for water from Jackfish Bay, Lake Superior, for 1991 (n = 192, median concentration 0.064 µg/L) (Comba *et al.*, 1993). The highest concentration of chloroform reported for the Niagara River at Fort Erie and Niagara-on-the-Lake from 1990 to 1993 was 0.19 µg/L (n = 293) (Environment Canada, 1996b). Concentrations of chloroform in 107 samples of Quebec surface waters collected from 1990 to 1993 ranged from below the 0.2 µg/L detection limit to 44 µg/L (MENVIQ, 1996). The detection limit in these various studies ranged from <0.001 to 1 µg/L. The median value of the reported chloroform concentrations from the four provinces was <0.2 µg/L (n = 984), while the 95th and 99th percentiles were <1 µg/L and 2.94 µg/L, respectively.

2.3.2.4 Soil and sediment

No monitoring studies of chloroform in soils and sediments have been conducted in Canada. Since chloroform does not appear to be sorbed in sediments or soils to any great extent, it is unlikely that the substance will accumulate to elevated levels in these media. This supposition is supported by the limited data available from studies outside of Canada (Table A.7 in Environment Canada, 1999a).

2.3.2.5 Groundwater

There have been cases of groundwater contaminated with chloroform from leachates in the immediate vicinity of waste disposal sites and landfills in Canada. These levels are not typical of groundwaters in Canada, even in industrialized areas, and are expected only near hazardous waste sites and landfills that are poorly contained. Concentrations of chloroform as high as 53 200 µg/L were detected in groundwater located approximately in the centre of the Special Waste Compound at a landfill site in the Ottawa, Ontario, area in 1981 (Jackson *et al.*, 1985). This Special Waste Compound received waste chemicals from 1969 to 1980. Remediation activities have been carried out at this landfill since 1991, and chloroform concentrations have decreased considerably. In 1998, the highest concentration of chloroform in groundwater from the same sampling site was 97.1 μg/L, while the concentration of chloroform at a sampling site approximately 50 m away was 5.8 μg/L (Moralejo, 1999). Concentrations as high as 950 μg/L in leachates from a chemical company landfill near Sarnia, Ontario (King and Sherbin, 1986), and 916 μg/L in the groundwater at Ville Mercier, Quebec (Pakdel *et al.*, 1992), have also been reported. Barker (1988) reported concentrations up to 25 μg/L in leachate-contaminated groundwaters from southern Ontario. Monitoring of leachates from a Muskoka, Ontario, landfill indicated chloroform levels ranging from below the detection limit (1 μg/L) in a collection trench sample to 2–3 μg/L in two culvert well samples taken in July 1986 (McBride *et al.*, 1987). Data on concentrations of chloroform in groundwater not associated with waste disposal sites and landfills are very limited. Reported chloroform concentrations in 31 groundwater samples collected in British Columbia in 1987 and 1989 were all below the 1 μg/L detection limit (B.C. MOE, 1996). Concentrations of chloroform in groundwater reported from the Lower Fraser Valley of British Columbia in 1992 and 1993 ranged from below the detection limit of 0.2 μg/L to 13.8 μg/L (Carmichael, 1996).

2.3.2.6 Biota

No monitoring studies on levels of chloroform in biota in Canada have been identified. As with soil and sediments, significant accumulation of chloroform in tissues is not expected given its low log K_{ow}, low bioconcentration factor and rapid metabolism.

2.3.2.7 Drinking water

Chlorine, the most commonly used primary and residual disinfectant in potable water treatment, can react with organic materials (e.g., humic and fulvic acids) that occur naturally in all surface water and groundwater supplies to form disinfection by-products (DBPs), including THMs. Chloroform is the principal DBP and THM in chlorinated drinking water (Lebel and Williams, 1995).

The chemistry of the reactions between chlorine and the organic materials present in water is complex and poorly understood; however, important factors include the type and concentrations of organic materials in the raw water, the chlorine reaction time, temperature and chlorination pH (Williams *et al.*, 1998). Consequently, there is a great degree of variation in the measured concentrations of chloroform in drinking water. Some specific contributors to the spatial and temporal variations in concentrations have been identified. Concentrations of organic materials in the raw water vary from one region to another, although the levels are generally higher in surface water than in groundwater. The extent of formation of chloroform varies with different water treatment processes (e.g., chlorine–chloramine, chlorine–chlorine, ozone–chlorine). Concentrations of chloroform in chlorinated water in treatment plants and distribution systems are approximately twice as high during summer months as during winter months, as a consequence of the higher concentrations of the precursor organic materials in the raw water during the warmer period. Levels can increase as the chlorinated water moves from the water treatment plant through the distribution system (Williams *et al.*, 1995). Further increases in concentrations of chloroform in water can occur in domestic hot water tanks (Benoit *et al.*, 1997).

The addition of dechlorinating preservatives to water samples lowers their pH and can have a significant effect on the measured concentrations of chloroform. In an investigation of optimum methodology for sample preparation, the most accurate determinations of the concentrations of chloroform in water samples at the time of their collection were achieved by adjusting the pH to 4.5–5.0 (e.g., with 0.1 N HCl) prior to the addition of the dechlorinating preservative (Lebel and Williams, 1995).

While information on levels of chloroform in water samples at the consumer tap would be preferred as a basis for estimation of human exposure, the majority of data concerning concentrations of chloroform in drinking water in Canada originates from samples collected within water treatment plants and their distribution systems. Data on concentrations in drinking water were obtained from Canadian provinces and territories for various periods as far back as 1985 and as recently as 1997 (Health Canada, 1999). In general, samples were preserved with sodium thiosulphate, without prior pH adjustment, and analysed by the purge-and-trap technique and gas chromatography with electron capture or mass selective detection. Limits of detection were generally between 0.1 and 1.0 µg/L. Concentrations of chloroform measured in drinking water during the 1990s are summarized in Table 2.

It is apparent from Table 2 that the mean and maximum concentrations of chloroform in drinking water were highest in Manitoba and Alberta. Consequently, the data from Manitoba and Alberta were pooled ($n = 2597$) to provide a distribution of concentrations for use in a reasonable worst-case (RWC) exposure scenario. Statistical parameters of this RWC data set are compared with those for the entire set of provincial/territorial data in Table 3.

National surveys have also been conducted, although numbers of samples were much smaller. In all cases, sample pH was adjusted at the time of collection, prior to addition of the dechlorinating preservative. During 1993, samples of water collected before and after treatment at the plant and in the distribution system were collected on two occasions (i.e., winter and summer) from 53 facilities in nine provinces. Chloroform was present at concentrations greater than the 0.2 µg/L limit of detection in all of the 214 samples collected (Williams *et al.*, 1995). The median and arithmetic mean concentrations were 13.4 µg/L and 27.6 µg/L, respectively, and the maximum concentration was 336 µg/L. Levels varied widely among the provinces, from a low arithmetic mean value of 6.5 µg/L (for $n = 8$ samples) in Newfoundland to a maximum mean concentration of 62.1 µg/L (for $n = 16$ samples) in Nova Scotia (Health Canada, 1999). Arithmetic mean and median concentrations of chloroform were approximately twice as high in samples collected during the summer months as in samples collected during the winter months. Similarly, the arithmetic mean and median concentrations of chloroform were about twice as high in samples collected from the approximate midpoints of the distribution systems as in samples collected in the water treatment plant, following disinfection but prior to distribution (Williams *et al.*, 1995).

Concentrations of DBPs for each of three water treatment plants located in or near the National Capital Region were examined as a function of water treatment practice, season and location in the distribution system. The three plants were selected to represent the three main treatment processes used in Canada (i.e., chlorine–chloramine, chlorine–chlorine, ozone–chlorine). Samples of raw water, treated water at the plant (i.e., after final disinfection, but before distribution) and treated water from three locations within the distribution systems (i.e., near plant, midpoint and end-of-line) were collected on a monthly basis throughout 1994 (Lebel *et al.*, 1996). The median and arithmetic mean concentrations (for n = 144 samples) were 29.1 µg/L and 33.9 µg/L, respectively, with a maximum value of 100 µg/L. The previously identified trends of seasonal variation (i.e., higher in warmer months) and increases in the concentrations with increasing distance from the treatment plants were clearly evident. The specific water treatment process was an important factor in determining the extent to which concentrations of chloroform increased as the treated water was transported through the distribution system (Lebel *et al.*, 1996).

The concentrations of chloroform in the incoming water supply in an experimental shower located in Burnaby, British Columbia, were twice as high in the summer as in the winter (Benoit *et al.*, 1997). However, storage in the hot water tank increased the level of chloroform twice as much in the winter as in the summer. The net result was that concentrations of chloroform in the warm water used for showering were relatively constant for both seasons (e.g., the concentration in the warm water entering the shower was approximately 24 µg/L when the incoming cold water contained approximately 6 µg/L [winter] or 12 µg/L [summer]; Benoit *et al.*, 1997).

The concentrations of chloroform in samples from 182 bottles of water in Canada collected during 1990 were determined (Dabeka *et al.*, 1992; Page *et al.*, 1993). Limits of detection ranged from 0.5 to 3.0 µg/L. Chloroform was not detected in any of 61 samples of mineral water and was detected in only 1 of 86 samples of spring water (at a concentration of 3.7 µg/L). Chloroform was detected in 10 of the remaining 35 samples, which included carbonated, demineralized, deionized, treated and distilled waters (Page *et al.*, 1993).

2.3.2.8 Food

The source or sources of chloroform in food are not clearly understood, although migration of chloroform from packaging solvents, glues and inks has been documented, and the possibility of transfer from surfaces cleaned with chlorinated water to lipid-containing foods contacting these surfaces has been identified. The use of chlorinated water by bottling plants (e.g., soft drink manufacturers) may explain the presence of chloroform in some beverages (Wallace, 1997). Chloroform introduced to foods as a consequence of the use of chlorinated drinking water during food preparation likely escapes by volatilization during cooking, reducing the concentrations in the table-ready foods.

In samples of various beverages (i.e., juices, soft drinks, milk) and dry foods (decaffeinated coffee and tea) purchased in Ottawa, Ontario, concentrations of chloroform were less than the limit of detection (i.e., 0.05 µg/kg) in all of the dry foods (Page and Lacroix, 1993). However, chloroform was detected in 11 of the 13 beverages sampled, at a maximum concentration of 14.8 µg/kg in a fruit drink. Subsequently, additional samples of foods (i.e., cream, flour, pastry mix, salad dressings, peanut butter, margarine, butter, vegetable oil) and beverages (i.e., coffees, fruit juices, soft drinks, milk) were purchased from supermarkets in Ottawa. Limits of detection could not be determined accurately (Page and Lacroix, 1995). Chloroform was detected in 41 of 47 samples of foods and beverages at concentrations ranging from 0.23 to 129 µg/kg. The highest three concentrations reported were in butter (i.e., 50, 83 and 129 µg/kg).

Groceries were purchased from four retailers in the Windsor, Ontario, area and were divided into 33 composite groups for analysis by headspace gas chromatography with mass selective detection (Enviro-Test Laboratories, 1992). Limits of detection were 1.0 µg/L for liquids and 5 µg/kg for solids. Chloroform was detected in 5 of the 33 composites (i.e., cheese/butter, canned meats, vine vegetables, soft drinks and dehydrated soups) at a maximum concentration of 67 µg/L. In a similar study conducted the following year by the same laboratory, groceries from four retailers in Ville Mercier, Quebec, were grouped into 35 composites (Enviro-Test Laboratories, 1993). Chloroform was detected in only two composite groups (i.e., soft drinks and alcohol).

There are also limited data on the concentrations of chloroform in food and beverages in the United States. McNeal *et al.* (1995) detected chloroform (at concentrations above 0.02 µg/kg) in 19 of 20 beverage samples purchased at markets in Washington, D.C., in 1991 and 1992. Chloroform was also detected in some of the food samples purchased at these outlets. Chloroform was detected in 94 of 231 table-ready food items obtained from the U.S. FDA's market basket collection (Daft, 1988a). Cheddar cheese contained the highest concentration (i.e., 312 µg/kg). Subsequently, Daft (1988b) detected chloroform in 16 of 22 food samples obtained from the U.S. FDA's Total Diet Study. Heikes and Hopper (1986) measured the concentrations of several fumigants, including chloroform, in whole grains, milled grain products and intermediate grain-based foods. Concentrations of chloroform ranged from 0.5 µg/kg in lasagna noodles to 3400 µg/kg in wheat.

Among food groups, the highest concentrations of chloroform have frequently been measured in dairy products. Dairy cows with access to chlorinated water or other sources of chlorine may store chloroform in fatty tissues, including milk fat (Wallace, 1997). Heikes (1987) detected chloroform in 10 of 18 table-ready food samples and measured a maximum concentration of 670 µg/kg in butter. Chloroform was present in concentrations ranging from 30 to 255 µg/kg in 36 samples of butter collected from 14 retail outlets in Washington, D.C. (Miller and Uhler, 1988). Heikes *et al.* (1995) analysed 234 table-ready foods and detected chloroform (at greater than the 5 µg/kg limit of detection) in 44 samples, including margarine (7.3 µg/kg), butter (38.9 µg/kg) and cream cheese (110 µg/kg).

Data from the United States and Canada were sufficient to serve as a basis for estimating the minimum, midpoint and maximum concentrations of chloroform for 131 of the 181 foods for which per capita daily intake rates (i.e., grams/day) are available for estimation of the daily intake of chemical substances from the ingestion of foods and beverages (EHD, 1998; Health Canada, 1999). Detectable concentrations were present in 79 of the 131 food items, while concentrations were less than the limits of detection in the remaining 52 items. The midpoint estimates of concentrations were greater than 100 µg/kg in 12 food items (i.e., butter, margarine, vegetable fats and oils, baby food cereal, pizza, marine fish, fresh fish, crackers, pancakes, veal, beef roast and cheese).

2.3.2.9 Consumer products

In the United States, emissions from approximately 5000 materials and assembled products in an enclosed chamber operated at elevated temperature (49°C) and reduced pressure (12 psia) were determined. A small number of these products emitted chloroform, usually in trace amounts (Ozkaynak *et al.*, 1987). Emissions of chloroform were detected (median emission levels are reported in parentheses) from the following materials (listed in decreasing order): ink and pen (10.0 µg/g), miscellaneous housewares (4.85 µg/g), photographic equipment (2.5 µg/g), rubber (0.9 µg/g), electrical equipment (0.23 µg/g), lubricant (0.2 µg/g), adhesives (0.15 µg/g), fabric (0.1 µg/g), paper (0.1 µg/g), photographic film (0.1 µg/g), tape (0.05 µg/g) and foam (0.04 µg/g).

Chloroform was detected (the reporting limit was 0.1% by weight, or 1000 µg/g) in only 3 of 1043 products in eight product classes purchased from stores in six U.S. cities. The product classes in which chloroform was detected were household cleaners/polishes, fabric and leather treatments, and miscellaneous products (Sack *et al.*, 1992).

The addition of chlorine bleaches or chlorine-containing detergents while washing clothes or dishes may create chloroform through their reaction with dirt and organic material, as demonstrated by Wallace *et al.* (1987) in an exposure chamber study.

A mixture of ferric oxide and chloroform has been routinely used during dental restoration as a disclosing medium to detect areas of interference when fitting crowns and dentures (Evans, 1995). Chloroform is also a component of root canal sealers used in dentistry (Allard and Andersson, 1992).

2.3.2.10 Human tissues and fluids

Data on concentrations of chloroform in human tissues and fluids in Canada were not identified. In the United States, concentrations ranging from 36.5 to 48.7 µg/L were measured in the urine of healthy male graduate students in New Jersey (Youssefi *et al.*, 1978). Chloroform was detected in 40 of 42 samples of breast milk from nursing mothers in five U.S. hospitals at concentrations ranging from 0.1 to 65 µg/L (Erickson *et al.*, 1980). It was also detected in 7 of 12 breast milk samples collected from nursing mothers in the United States; however, the concentrations were not quantified, and a limit of detection was not reported (Pellizzari *et al.*, 1982). Chloroform was detected (limits of detection ranging from 2 to 100 µg/kg) in 36 of 46 samples of human adipose tissue collected post-mortem in the United States in 1981 and 1982. The mean and maximum concentrations were 44 µg/kg and 580 µg/kg, respectively (U.S. EPA, 1986). Chloroform was detected (i.e., above a limit of detection of 0.1 µg/L) in 54% of 979 samples of human blood collected from across the United States, but concentrations were not quantified (Ashley *et al.*, 1994).

In the United Kingdom, concentrations of chloroform were measured in post-mortem tissue samples from four male and four female subjects ranging in age from 48 to 82 years. Concentrations of chloroform in body fat ranged from 5 to 68 µg/kg, with a mean concentration of 51 µg/kg. Mean concentrations were lower in the liver, kidney and brain (McConnell *et al.*, 1975). Chloroform was detected (limits of detection not reported) in more than 80% of blood samples collected from rural and urban locations in Italy, at concentrations ranging from 0.025 to 7.6 µg/L (Brugnone *et al.*, 1994). Mean concentrations were similar in rural (0.62 µg/L for n = 127) and urban (0.66 µg/L for n = 107) residents.

2.3.2.11 Showers/baths

Since the mid-1980s, domestic tap water has been recognized as a potentially important source of VOCs in indoor air. Common domestic water uses (e.g., showering, bathing, toilet use, washing dishes, washing clothes, food preparation and cooking, etc.) involve far greater volumes of water daily than that ingested. Absorption of chemical contaminants through the skin may also occur during many of these uses of domestic water. Consequently, exposure to volatile components of tap water through inhalation and/or dermal absorption may be equal to or greater than exposure through ingestion of tap water from the same source (Shimokura *et al.*, 1998).

The vapour pressure (i.e., 26.2 kPa at 25°C) and Henry's law constant (i.e., 314 $Pa{\cdot}m^3/mol$) for chloroform are such that its volatilization from water is likely (Andelman, 1985). Based on other physical-chemical properties (e.g., $\log K_{ow} = 1.97$ and relatively low molecular weight of 119.38 g/mol), dermal absorption may occur when the skin contacts water containing dissolved chloroform.

In addition, concentrations of THMs, including chloroform, increase when water is heated, such as in domestic hot water tanks (Weisel and Chen, 1994; Benoit *et al.*, 1998). Although compounds such as chloroform would normally be expected to volatilize from water as it is heated (e.g., as in boiling water for preparation of beverages), this cannot occur in a domestic hot water tank and associated plumbing, since this is a closed system, with no headspace.

Among the various scenarios involving exposures to chloroform in chlorinated water via routes other than ingestion, the focus of most studies has been showering. Showers are especially effective, compared with other typical indoor uses of tap water, in releasing VOCs to indoor air, due to agitation and aeration as the water is run (Andelman, 1985). They also occur frequently (e.g., daily) for many members of the general population and involve greater volumes of water and more extensive dermal contact than do other common indoor uses of tap water. However, due to the dynamic nature of a shower, it is difficult to estimate accurately the concentrations of chloroform in the air breathed while showering or in the water contacting the skin. As chloroform volatilizes from the heated water entering through the shower head, the concentration of chloroform in the breathing zone of a showering individual increases in a time-dependent, but non-linear, manner. As the chloroform volatilizes from the incoming water, the concentration of chloroform in the water is decreased. Therefore, water contacting the skin has a lower concentration of chloroform than water entering through the shower head.

There have been several different approaches to estimating the levels of chloroform to which individuals may be exposed when showering. Some researchers (e.g., Jo *et al.*, 1990a,b; Giardino and Andelman, 1996; Kuo *et al.*, 1998) have measured the concentrations of chloroform in the air of a shower compartment and tried to relate this to levels in the incoming water. Water temperature and flow rate and the air exchange rate between the shower compartment and adjacent area (i.e., bathroom) are important parameters affecting these relationships. Alternatively, experimental data have been developed concerning the transfer efficiency of chloroform from shower water to air (e.g., Little, 1992; Tancrède *et al.*, 1992; Giardino and Andelman, 1996; Keating *et al.*, 1997). Based on these data, one-half or more of the chloroform entering through the shower head is volatilized into the air under typical shower operating conditions (i.e., water temperature and flow rate, air exchange rate).

In other studies, there have been attempts to characterize exposure to chloroform while showering through its determination in exhaled breath following "normal" showers and during inhalation-only and dermal-only exposures. Inhalation-only exposures are assessed by having volunteers shower in waterproof clothing (e.g., rubber clothes and boots). Dermal-only exposures are assessed by having volunteers shower without clothes while breathing purified air. Jo *et al.* (1990a) observed that for normal and inhalation-only showers, concentrations of chloroform in exhaled breath tended to increase with the concentration of chloroform in water and concluded that inhalation and dermal absorption contributed approximately equally to the internal chloroform dose. These conclusions were confirmed by Weisel and Jo (1996) in similar experiments involving solely dermal-only rather than normal showers.

In more refined studies conducted by Benoit *et al.* (1998), the breath sampling technique was improved so that concentrations in exhaled alveolar breath could be measured. Breath samples were collected more frequently during and after showering, to better characterize uptake and elimination. Shower air samples were collected directly from the breathing zone, to minimize the effects of non-homogeneity of concentrations of chloroform in the air of the shower compartment. Additional details of this work are presented in Health Canada (1999). Benoit *et al.* (1998) expressed the amounts of chloroform taken up as equivalent volumes of cold water ingested. Preliminary results, based on four volunteers, suggested that showering for 10 minutes with warm water (i.e., 40°C) that has been treated with a chlorinated disinfectant is equivalent on an annual average to drinking 2.7 L of cold water from the same water supply. Dermal absorption accounted for an average of 30% of the total uptake. Comparable estimates of the amounts of chloroform absorbed through the skin during a 10-minute shower, as a function of different concentrations of chloroform in the tap water supply, were developed from empirical relationships (Health Canada, 1999). These estimates were based on an experimentally determined human skin permeation coefficient for chloroform in water (i.e., $K_p = 0.16$ cm/h) from Nakai *et al.* (1999) using the non-steady-state approach recommended in U.S. EPA (1992).

It was noted that the dose of chloroform from showering for longer periods would increase by a squared function, since both the level of exposure and the exposure duration are increased (i.e., doubling the time would quadruple the dose) (Benoit *et al.*, 1998). The level of exposure would increase since the concentration of chloroform in the air of a shower compartment would continue to increase throughout the duration of a shower, due to the constant addition of chloroform volatilizing from the incoming heated water.

In view of the limited focus of currently available experimental data sets on measured levels of chloroform in the air of showers or exhaled breath during showering, for the present assessment, estimates of the average concentration of chloroform in the air of a shower compartment during a 10-minute shower were developed based on measured concentrations of chloroform in drinking water supplies across Canada (Health Canada, 1999). Lower estimates were based on an assumed concentration of chloroform of 50 µg/L, which is approximately the mean concentration of chloroform in water in Canada (i.e., 47.3 µg/L, according to provincial/territorial data; see Table 3). Assuming water flow rates of 5 or 10 L/min, a water temperature of 40°C, a transfer efficiency of 50%, a 10-minute shower duration and minimum air exchange, the range of average concentrations of chloroform in the air of the shower compartment was estimated as 417–833 µg/m^3. With similar assumptions, but a concentration of chloroform in water of 166 µg/L (i.e., the 95th percentile of the distribution of concentrations, according to provincial/territorial data; see Table 3), the range of average concentrations of chloroform in the air of the shower compartment was estimated as 1382–2765 µg/m^3 (Health Canada, 1999). While there are limitations of this approach (see Section 3.3.5), it takes into account the considerable variability in levels of chloroform in water supplies across Canada.

Some additional inhalation exposure typically occurs as an individual step from the shower compartment into the adjacent area (i.e., bathroom), since concentrations of chloroform in the air would be elevated due to the proximity of the source (i.e., the shower). The degree of air exchange between the bathroom and adjacent areas of the home and the operation of a bathroom exhaust fan are important factors determining the extent of this additional inhalation exposure. It is also possible (e.g., Wallace, 1997) that a second individual showering soon after the first individual has finished could receive relatively higher exposures, as concentrations of chloroform in the air of the shower compartment may not have returned to background levels.

Relatively fewer data are available concerning exposure to chloroform by inhalation and dermally while bathing. Overall, somewhat less total intake may occur during bathing than during showering, since adults bathe less frequently than they shower (U.S. EPA, 1997). Moreover, a bath faucet is less efficient than a shower head at stripping chloroform from water, and the water contacting the skin is not being constantly replaced by "newer" water (as in a shower). On the other hand, the average duration of baths is typically longer than the average duration of showers

(U.S. EPA, 1997), resulting in longer periods of both inhalation and dermal exposure to chloroform. No data were available on the concentrations of chloroform in the air of a bathtub enclosure or in the breathing zone of a bather as a function of the concentration of chloroform in the water in the tub. Therefore, no estimates of intake by inhalation during bathing could be derived. Consequently, the total intakes of chloroform during typical baths and showers are currently assumed to be approximately equal.

2.3.2.12 Indoor pools

The general population is also exposed to chloroform by inhalation and dermally while swimming in indoor swimming pools, due to reaction primarily between the chlorine added (e.g., as sodium hypochlorite) and organic matter (e.g., sweat, urine, cosmetic residue). While data on chloroform levels in swimming pools in Canada were not identified, concentrations of chloroform as high as 980 µg/L in indoor swimming pool water have been reported. However, in the most recent studies conducted in the United States, the concentrations have been in the range of 24–150 µg/L (Weisel and Shepard, 1994; Wilson, 1995; Lindstrom *et al.*, 1997).

Short-term concentrations of chloroform in the air immediately above the surface of the water in indoor swimming pools may be as high as 650 $\mu g/m^3$. In the most recent studies conducted in the United States, however, the concentrations have ranged from 13 to 150 $\mu g/m^3$ (Weisel and Shepard, 1994; Wilson, 1995; Lindstrom *et al.*, 1997). Concentrations in the air in the building containing an indoor swimming pool are highly variable, generally decreasing with increasing distance from the water surface. They are also affected by the concentration of chloroform in the pool water, the water temperature, the air temperature, air circulation, the turbidity of the water and the number of swimmers in the pool (Lahl *et al.*, 1981; Armstrong and Golden, 1986; Jo, 1994).

The levels of chloroform measured in blood plasma and exhaled breath have been consistently higher in swimmers than in subjects with no exposure to indoor pools (Health Canada, 1999). Levels of chloroform in swimmers' plasma samples increased with the level of exertion and were most closely correlated with the concentration of chloroform in the air and the length of time spent swimming (Aggazzotti *et al.*, 1990).

Wilson (1995) reported concentrations of chloroform in exhaled breath of 48.3, 15.7 and 4.9 $\mu g/m^3$, respectively, for normal, inhalation-only and dermal-only exposures and concluded that the majority of the intake of chloroform by swimmers was by inhalation, rather than by dermal absorption. In the only Canadian study identified, Lévesque *et al.* (1994) measured concentrations of chloroform in the breath of normal swimmers and swimmers wearing scuba gear (i.e., exposed dermally only). When the swimmers were exposed to high concentrations of chloroform in the pool water and air, it was estimated that 78% of the body burden after 55 minutes of exposure was due to inhalation, and the remaining 22% was due to dermal uptake. Relatively less dermal absorption while swimming in indoor pools than while showering or bathing is consistent with the premise that dermal absorption becomes more significant as the water temperature increases.

Competitive swimmers are potentially most highly exposed to chloroform due to the high level of physical exertion (and higher breathing rate) and higher frequency of this activity (i.e., while training). However, the estimated intakes vary widely (Health Canada, 1999).

Limited available data indicate that dermal absorption may be more significant for users of hot tubs than for swimmers in indoor pools, due to the relatively higher water temperature in a hot tub (Wilson, 1995).

2.4 Effects characterization

2.4.1 Ecotoxicology

Chloroform induces a narcosis type I mode of toxic action (Sixt *et al.*, 1995). The log K_{ow} is a key parameter for narcosis type I compounds because it describes the tendency for transport across cellular membranes to the site of action. Narcosis type I is the least toxic mode of action and is often referred to as "baseline toxicity."

A brief summary is presented below of the most sensitive reported effects of chloroform on terrestrial and aquatic organisms.

2.4.1.1 Terrestrial organisms

Only one study on the toxicity of chloroform to terrestrial microorganisms was identified. Walton *et al.* (1989) reported that chloroform applied once at a rate of 1000 mg/kg to a silty loam soil caused microbial respiration to increase for several days (e.g., day 4: CO_2 efflux$_{treatment}$/CO_2 efflux$_{control}$ = 1.39) before returning to control levels 6 days after treatment. The same treatment applied to sandy soils caused an initial depression in microbial respiration followed by a stimulation period (e.g., day 4: CO_2 efflux$_{treatment}$/CO_2 efflux$_{control}$ = 1.77) and a return to control levels 6 days after treatment.

The toxicity of chloroform to terrestrial invertebrates has been examined in few studies. Two of the available studies (Neuhauser *et al.*, 1985, 1986; Callahan *et al.*, 1994) were contact tests with the earthworm, *Eisenia fetida*. The exposure conditions used in these contact tests (chloroform added to filter paper), however, are not directly relevant for estimating potentially harmful concentrations in the soil. The other available study (Alphei and Scheu, 1993) demonstrated that fumigation treatments with chloroform (concentration not stated) eliminated protozoans from the soil but did not eliminate microbial or nematode (roundworm) populations. As with the contact tests, the experimental exposure conditions are not considered relevant for estimating potentially harmful concentrations in the soil.

No information on the toxicity of chloroform to birds or wild mammals was identified. Data on the effects of chloroform on experimental animals are presented in some detail in Section 2.4.3. Adverse effects were seen in inhalation studies at chloroform concentrations as low as 2 ppm (9.8 mg/m^3). At that concentration, cellular proliferation occurred in nasal passages in rats and mice (Larson *et al.*, 1996; Templin *et al.*, 1996b).

2.4.1.2 Aquatic organisms

There is information on the toxicity of chloroform to a number of aquatic organisms, including bacteria, algae, invertebrates, fish and amphibians.

Among aquatic organisms, amphibians appear to be quite sensitive to chloroform. The toxicity of chloroform to early life stages of seven species of amphibians was determined by Birge *et al.* (1980) and Black *et al.* (1982). The 4-day post-hatching LC_{50}s ranged from 0.27 mg/L in spring peepers (*Hyla crucifer*) to >68 mg/L in African clawed frogs (*Xenopus laevis*). The 4-day post-hatching LC_1 and LC_{10} values for spring peepers were 0.0019 and 0.0177 mg/L, respectively. The LC_{50} and LC_{10} values for the second most sensitive amphibian (the leopard frog, *Rana pipiens*) were 4.16 and 0.383 mg/L, respectively (Birge *et al.*, 1980). Birge *et al.* (1980) reported that spring peepers were consistently one of the most sensitive species in toxicity tests on 11 organic compounds. They speculated that amphibians (such as spring peepers) that are restricted to a narrow range of ecological conditions (i.e., aquatic) are more sensitive than those that can tolerate a wide range of conditions (i.e., aquatic, semi-aquatic, terrestrial).

Microorganisms can also be quite sensitive to chloroform. Jackson and Brown (1970) reported that a concentration of 0.1 mg chloroform/L could retard anaerobic digestion of sewage sludge. The study did not provide a rationale for this value, nor did it predict how much inhibition could be expected. Yang and Speece (1986) observed inhibition of unacclimated cultures at 0.5 mg/L; with acclimation, concentrations up to 15 mg/L could be tolerated.

Several flow-through studies have been carried out to determine the effects of chloroform on fish. In rainbow trout, 4-day post-hatching LC_{50}s range from 1.24 mg/L (200 mg $CaCO_3$/L) to 2.03 mg/L (50 mg $CaCO_3$/L) (Birge *et al.*, 1979). With bluegills, the 96-hour LC_{50} in a flow-through study was 18.2 mg/L (Anderson and Lusty, 1980). In a flow-through test with measured concentrations, Pearson and McConnell (1975) observed a 96-hour LC_{50} of 28 mg/L in dab (*Limanda limanda*) in the only available acute toxicity test for marine fish species.

Among aquatic invertebrates, the rotifer, *Brachionus calcyciflorus*, was particularly sensitive, with a 1-hour LC_{50} of 2 mg/L (Snell *et al.*, 1991). For *Daphnia magna*, 48-hour LC_{50}s range from 28.9 mg/L (U.S. EPA, 1978) to 353 mg/L (Cowgill and Milazzo, 1991), with most results in the lower end of the range. Using growth of *D. magna* as an endpoint, Hermens *et al.* (1985) reported a 16-day EC_{50} of 59.8 mg/L, with a 16-day No-Observed-Effect Concentration (NOEC) of 15 mg/L.

Freshwater and marine algae appear to be relatively insensitive to chloroform exposure. Bringmann and Kühn (1977, 1978) observed an initial reduction in cell multiplication of *Microcystis aeruginosa* at 185 mg/L during a 6-day exposure to chloroform. A similar result was observed by Kühn and Pattard (1990) using the green alga, *Scenedesmus subspicatus* (48-hour

EC_{10} for biomass, 225 mg/L; EC_{50}, 560 mg/L). Reported EC_{50}s for endpoints such as cell count, biomass and carbon dioxide uptake (photosynthesis) range from 382 mg/L for *Chlamydomonas angulosa* (Hutchinson *et al.*, 1980) to >1000 mg/L for *Selenastrum capricornutum* (Cowgill *et al.*, 1989). In the only available test for vascular plants, Cowgill *et al.* (1991) observed no effects within the concentration range 28–1000 mg/L on two duckweed species, *Lemna gibba* and four clones of *Lemna minor*.

2.4.2 Abiotic atmospheric effects

The potential for chloroform to contribute to the depletion of stratospheric ozone, climate change and the formation of ground-level ozone has been examined.

The Ozone Depletion Potential (ODP) for chloroform may be calculated to be 0.0326 (relative to the reference compound CFC-11, which has an ODP of 1) using the formula:

$$ODP_{chloroform} = (t_{chloroform}/t_{CFC\text{-}11}) \times (M_{CFC\text{-}11}/M_{chloroform}) \times [(n_{Cl} + \alpha n_{Br})/3]$$

where:

- $t_{chloroform}$ is the atmospheric lifetime of chloroform (620 days, or 1.7 years, based on the maximum estimated half-life for chloroform in the troposphere; Singh *et al.*, 1978),
- $t_{CFC\text{-}11}$ is the atmospheric lifetime of CFC-11 (60 years),
- $M_{CFC\text{-}11}$ is the molecular mass of CFC-11 (137.5 g/mol),
- $M_{chloroform}$ is the molecular mass of chloroform (119.4 g/mol),
- n_{Cl} is the number of chlorine atoms in the chloroform molecule (3),
- n_{Br} is the number of bromine atoms in the chloroform molecule (0), and
- α is a measure of the effectiveness of bromine in ozone depletion with respect to chlorine (~30) (Environment Canada, 1997a).

Because the ODP exceeds zero, there is justification for determining the potential for chloroform in the atmosphere to cause a decline in worldwide stratospheric ozone levels.

Estimating the risks posed by chloroform to the stratospheric ozone layer requires realistic estimates of tropospheric half-lives, as well as information on the transport of chloroform and its breakdown products to and from the stratosphere. Depending on the rate constant chosen for the reaction of hydroxyl radicals with chloroform, temperature, hydroxyl radical concentration, latitude and other factors, chloroform half-lives in the troposphere could vary from 54.5 to 620 days. Assuming an atmospheric half-life of 193 days, Kindler *et al.* (1995) predicted that 1.7% of the chloroform in the troposphere would migrate to the stratosphere, where its half-life would be 3.18 years. Considering both the photochemical reactions and transport of chloroform and its major breakdown product, phosgene, in the atmosphere, Kindler *et al.* (1995) estimated that 1–1.8% of the chlorine in chloroform molecules released at the earth's surface is transported into the stratosphere as reactive chlorine (the corresponding values for CFC-11 range from 93.2% to 100%). Therefore, the net chlorine loadings to the stratosphere from chloroform and its degradation products are small. Kindler *et al.* (1995) estimated a more realistic stratospheric ODP for chloroform of 0.0083. This value includes the effect of a downward transport of a significant fraction (up to 40%) of the stratospheric phosgene to the troposphere, where it is removed from the atmosphere by clouds. Chloroform would therefore not be an effective agent of stratospheric ozone depletion.

The Global Warming Potential (GWP) was calculated to be 0.0326 (relative to the reference compound CFC-11, which has a GWP of 1), based on the following formula:

$$\mathrm{GWP} = (t_{\mathrm{chloroform}}/t_{\mathrm{CFC\text{-}11}}) \times (M_{\mathrm{CFC\text{-}11}}/M_{\mathrm{chloroform}}) \times (S_{\mathrm{chloroform}}/S_{\mathrm{CFC\text{-}11}})$$

where:

- $t_{\mathrm{chloroform}}$ is the lifetime of chloroform (1.7 years),
- $t_{\mathrm{CFC\text{-}11}}$ is the lifetime of CFC-11 (60 years),
- $M_{\mathrm{CFC\text{-}11}}$ is the molecular weight of CFC-11 (137.5 g/mol),
- $M_{\mathrm{chloroform}}$ is the molecular weight of chloroform (119.38 g/mol),
- $S_{\mathrm{chloroform}}$ is the infrared absorption strength of chloroform (2389/cm^2 per atmosphere, default), and
- $S_{\mathrm{CFC\text{-}11}}$ is the infrared absorption strength of CFC-11 (2389/cm^2 per atmosphere).

The Photochemical Ozone Creation Potential (POCP) was estimated to be 8.14×10^{-3} (relative to the value of an equal mass of the reference compound ethene, which has a POCP of 100), based on the following formula:

$$\mathrm{POCP} = (k_{\mathrm{chloroform}}/k_{\mathrm{ethene}}) \times (M_{\mathrm{ethene}}/M_{\mathrm{chloroform}}) \times 100$$

where:

- $k_{\mathrm{chloroform}}$ is the rate constant for the reaction of chloroform with OH radicals (2.95×10^{-13} cm^3/mol per second),
- k_{ethene} is the rate constant for the reaction of ethene with OH radicals (8.5×10^{-12} cm^3/mol per second),
- M_{ethene} is the molecular weight of ethene (28 g/mol), and
- $M_{\mathrm{chloroform}}$ is the molecular weight of chloroform (119.38 g/mol).

These figures suggest that the potential contribution of chloroform to climate change and ground-level ozone formation is negligible (Bunce, 1996). The magnitude of these effects would depend on the concentration of chloroform in the atmosphere, and, in Canada, concentrations of chloroform in air are very low, usually less than 1 μg/m^3 (Section 2.3.2.1).

2.4.3 Experimental animals and in vitro

The content of these sections has been prepared principally on the basis of information included in WHO (1994) and ILSI (1997). Where considered necessary, documentation on key studies referenced in these reports has been expanded in the supporting documentation for this assessment; detailed results of more recent studies are also presented therein (Health Canada, 1999). Due to the extensive database on the toxicity of chloroform, only an overview of the available information is provided here. More detailed descriptions included are restricted to those for critical investigations relevant to the exposure–response analyses. For more detailed accounts of the relevant studies, supporting documentation should be consulted (WHO, 1994; ILSI, 1997; Health Canada, 1999).

In repeated-dose toxicity studies, effects of chloroform at lowest concentrations in rats and mice have been those associated with cytotoxicity and resulting regenerative proliferation in the proximal tubules of the kidney, the centrilobular region of the liver and the ethmoid turbinates of the nose. Measures of this toxicity include histopathological effects, release of hepatic enzymes and increased labelling indices. The results of shorter-term studies in which regenerative proliferation has been examined primarily to investigate mode of induction of cancer are summarized in Section 2.4.3.7.

2.4.3.1 Acute toxicity

Acute exposure to high doses of chloroform can result in death, usually due to liver damage, with the exception of male mice of very sensitive strains, whose death is caused by kidney damage. The higher susceptibility to acute toxicity of chloroform in these strains of mice (such as DBA, C3H, C3Hf, CBA, BALB/c, C3H/He) compared with other strains is genetically controlled. An absolute sex-related difference with respect to kidney damage, but not liver damage, has been described in mice. This is independent of the strain. Female mice do not develop renal lesions, although an increase in renal labelling index has been reported in female B6C3F1 mice in a 4-day drinking water assay (Larson *et al*., 1994a).

Some influence of age on acute toxicity of chloroform in rats has also been described (Kimura *et al*., 1971). The vehicle in gavage administration affects toxicity; corn oil vehicle enhanced the severity of nephrotoxicity in rats, compared with Emulphor and Tween-85 (Raymond and Plaa, 1997).

For the rat, LD_{50} values range from 450 to 2000 mg chloroform/kg-bw; in this species, there was no sex difference in susceptibility (Kimura *et al*., 1971; Chu *et al*., 1980). For OF1 female mice, an LC_{50} value of 6150 mg chloroform/m^3 (6-hour exposure) was reported by Gradiski *et al*. (1978).

A dose of 3070 mg chloroform/kg-bw in mineral oil administered to rats resulted in death due to central nervous system depression within minutes, and a dose of 980 mg chloroform/kg-bw resulted in hepatic centrilobular necrosis (Reynolds and Yee, 1967). When administered to newborn rats, chloroform was lethal at oral doses of 1500 mg/kg-bw; lower doses were not administered (Kimura *et al.*, 1971).

Hepatic necrosis was observed in male mice 48 hours following a single gavage administration of 240 mg/kg-bw (Reitz *et al.*, 1982). Minimal centrilobular enlargement was observed in male mice 4 days following intragastric administration of 66 mg/kg-bw (Moore *et al.*, 1982).

Both Osborne-Mendel and F344 rats had lesions in the ethmoid region of the nasal passage 48 hours following a single administration of 90 mg/kg-bw by gavage in corn oil, accompanied by increased BrdU-positive epithelial cells in the endoturbinate (Templin *et al.*, 1996a).

2.4.3.2 Short-term toxicity

Gavage administration to mice has resulted in decreased humoral immunity and increased relative liver weight at 50 mg/kg-bw per day (Lowest-Observed-Adverse-Effect Level, or LOAEL) for 14 days (Munson *et al.*, 1982). Mice exposed by gavage to 37 mg/kg-bw per day (LOAEL) for 14 days had mineralization, hyperplasia and cytomegaly in kidney and inflammation in liver (Condie *et al.*, 1983). Rats exposed to 11 mg/kg-bw per day in drinking water for 28 days had a decrease in neutrophils (Chu *et al.*, 1982a,b).

Female F344 rats had lesions in the peripheral olfactory epithelium after four daily doses of 34 mg/kg-bw in corn oil by gavage, accompanied by increased proliferation in olfactory epithelium (Larson *et al.*, 1995a); after 3 weeks of administration, these effects were observed only at 100 mg/kg-bw per day. Dorman *et al.* (1997) also exposed female F344 rats by gavage in corn oil and reported lesions in the ethmoid region of the nasal passages after five exposures to 34 mg/kg-bw per day. After 3 weeks' exposure, lesions were observed at 100 mg/kg-bw per day; but not at 34 mg/kg-bw per day.

Following 4 days' exposure of male F344 rats for 6 hours per day to 10 ppm (49 mg/m^3), minimal to mild lesions were observed in the ethmoid region of the nose, although proliferation was increased at 2 ppm (9.8 mg/m^3) (Templin *et al.*, 1996b). Following inhalation exposure to 10 ppm (49 mg/m^3) for 6 hours per day for 7 consecutive days, male F344 rats had lesions in nasal turbinates (Larson *et al.*, 1994b). Increased cell proliferation in central, proximal and distal regions of the first endoturbinate was reported in male F344 rats exposed by inhalation for 6 hours per day for 7 consecutive days to 10 ppm (49 mg/m^3), accompanied by histological changes and a significant increase in the width of the central turbinate bone (Mery *et al.*, 1994).

Larson *et al.* (1994b) observed no histological lesions in the nasal passages of female B6C3F1 mice exposed to up to 288 ppm (1411 mg/m^3) for 6 hours per day for 7 consecutive days. Cell proliferation was not measured. Mery *et al.* (1994) exposed female B6C3F1 mice to chloroform for 6 hours per day for 7 consecutive days and observed increased proliferation in the first endoturbinate at 10 ppm (49 mg/m^3).

2.4.3.3 Subchronic toxicity

Bull *et al.* (1986) reported histological changes in the liver of B6C3F1 mice receiving 60–270 mg/kg-bw per day in corn oil, but not with the same dose levels in Emulphor for 90 days. For corn oil administration, the LOAEL in both sexes was 60 mg/kg-bw per day, based upon increased absolute and relative liver weights and vacuolation and lipid accumulation in the liver. For Emulphor vehicle, at 60 mg/kg-bw per day (LOAEL), absolute and relative liver weights were increased in females only. Female CD1 mice exposed by gavage in Emulphor to 50 mg/kg-bw per day (LOAEL) for 90 days had increased liver weight and increased hepatic microsomal activity (Munson *et al.*, 1982). When female B6C3F1 mice were exposed to chloroform in drinking water for 90 days, fatty changes in the liver were observed at 263 mg/kg-bw per day (LOAEL) (U.S. EPA, 1980). In male Osborne-Mendel rats, cholesterol was significantly increased at 81 mg/kg-bw per day (Lowest-Observed-Effect Level, or LOEL) in a 90-day study in which chloroform was administered in drinking water (U.S. EPA, 1980). In a 6-month inhalation assay (Torkelson *et al.*, 1976), male rats (strain not specified) exposed to 123 mg/m^3 (LOAEL) had increased relative kidney weight and focal necrosis in the liver.

One of the investigations in which effects on the liver were reported at lowest concentration/dose was that by Heywood *et al.* (1979), in which chloroform was administered in a toothpaste base in gelatin capsules to male and female beagle dogs for 6 days per week for 7.5 years at dose levels of 15 or 30 mg/kg-bw per day. Sacrifice followed an observation period of 19–23 weeks, during which the chloroform treatment was withdrawn. The protocol included vehicle controls, untreated controls and those receiving alternative (non-chloroform) toothpaste. Each group contained 8 animals of each sex, with the exception of the vehicle control group, which included 16 of each sex. At the high dose, there were significant increases in serum glutamate–pyruvate transaminase (SGPT) levels at 6 weeks of treatment. At the low dose, significant increases in SGPT levels were observed at 34 weeks and after. Similar effects were not observed in the vehicle control or untreated control groups. "Fatty cysts" of the liver were observed in both dose groups at the end of the study; data on incidence are presented in Table 4. There were no treatment-related increases in tumours.

Larson *et al.* (1996) exposed female B6C3F1 mice for 6 hours per day for periods ranging from 4 days to 13 weeks. After 4 days, mild to minimal nasal lesions were observed at 10 ppm (49 mg/m^3), accompanied by increased proliferation in nasal turbinate lamina propria. After longer durations of exposure, histological changes in the nose were minimal at concentrations up to 90 ppm (441 mg/m^3), at which concentration increased cell proliferation persisted. Daily exposure of male F344 rats for 6 hours per day for 13 weeks resulted in mild histological changes in nasal passages at 2 ppm (9.8 mg/m^3) and increased proliferation at 10 ppm (49 mg/m^3) (Templin *et al.*, 1996b).

2.4.3.4 Chronic toxicity and carcinogenicity

In carcinogenicity bioassays in rats and mice following exposure both orally and by inhalation, chloroform has been carcinogenic in the mouse liver and in the male rat and male mouse kidney. The carcinogenic response has varied with different routes and vehicles of exposure and among sexes, species and strains. Summaries of tumour incidence in the identified carcinogenesis bioassays are presented in Table 5 for the liver and Table 6 for the kidney.

2.4.3.4.1 Liver

Chloroform is carcinogenic in the male and female mouse liver (NCI, 1976), but only following gavage in a corn oil vehicle. It was not carcinogenic in the liver of mice exposed in drinking water (Jorgenson *et al.*, 1985), although the daily doses were similar to those administered by corn oil gavage (NCI, 1976). Similarly, it was not carcinogenic by inhalation, despite an exposure escalation strategy that achieved final concentrations that were several-fold greater than those considered acutely lethal (Yamamoto, 1996).[1] Thus, neither the daily dose nor the cumulative dose of chloroform was predictive of tumour outcome following exposure in drinking water.

In a single study, there was an increased incidence of liver tumours in female Wistar rats following administration of chloroform in drinking water. The control group in this investigation was small. Moreover, the longer survival of the exposed females (185 weeks) compared with the control females (145 weeks) complicates interpretation of the results, since there was no baseline for comparison of the incidence of late-developing tumours in the treated groups (Tumasonis *et al.*, 1985, 1987). In other studies in which chloroform was administered in drinking water, by gavage in corn oil or by inhalation to various strains of rats, the incidence of liver neoplasia was not increased (NCI, 1976; Palmer *et al.*, 1979; Jorgenson *et al.*, 1985; Yamamoto, 1996).

2.4.3.4.2 Kidney

Chloroform has induced renal tumours in both rats and mice, but only in males. Renal tubular cell tumours were observed in mice exposed to chloroform by inhalation (Yamamoto, 1996) or in toothpaste preparations (Roe *et al.*, 1979) and in rats exposed by corn oil gavage (NCI, 1976) or in drinking water (Jorgenson *et al.*, 1985). The responses varied with route of exposure, administration vehicle and strain. However, it is difficult to draw meaningful conclusions regarding potential differences among strains on the basis of available data.

There was a significant increase in renal tumours in male BDF1 mice following inhalation (Yamamoto, 1996) and in male ICI mice exposed in either toothpaste or arachis oil (Roe *et al.*, 1979). However, there were no renal tumours in male B6C3F1 mice following exposure to chloroform by corn oil gavage (NCI, 1976) or in drinking water (Jorgenson *et al.*, 1985). Responses were positive in Osborne-Mendel rats (drinking water) (Jorgenson *et al.*, 1985) but not in F344 rats (inhalation) (Yamamoto, 1996) or Sprague-Dawley rats (toothpaste) (Palmer *et al.*, 1979).

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[1] These results have been published in summary form by Nagano *et al.* (1998).

The bioassay in which tumours were observed at lowest concentration or dose following exposure in a manner similar to that of humans (i.e., continuously in drinking water or by inhalation), namely kidney tumours in male rats, was that of Jorgenson *et al.* (1985). In this bioassay, male Osborne-Mendel rats were exposed to 0, 200, 400, 900 or 1800 mg chloroform/L drinking water (number of animals: 330, 330, 150, 50 and 50, respectively) for a period of 2 years. These concentrations (monitored by analysis) corresponded to time-weighted average daily doses of 0, 19, 38, 81 and 160 mg/kg-bw (Jorgenson *et al.*, 1985). Matched controls received an amount of water without chloroform equal to that consumed by the 1800 mg/L group. Clinical chemistry indicated renal impairment in control animals but not in the groups receiving 900 or 1800 mg/L. Renal impairment in the 200 and 400 mg/L dose groups was mild. These results are consistent with the occurrence of severe chronic nephropathy in the control animals associated with caloric overload on an *ad libitum* diet and a protective effect of dietary restriction in the high-dose exposed groups associated with reduced consumption of drinking water. Consistent with these results, mortality was decreased in the matched control group and inversely related to concentration of chloroform in the exposed groups. Data on organ weights were not provided. The only clear dose-related effect was an increase in renal tubular cell adenomas and adenocarcinomas, with combined incidence being significantly increased at the top dose. The incidence of tubular cell adenomas and adenocarcinomas (combined) was 4/301, 1/50, 4/313, 4/148, 3/48 and 7/50 ($p < 0.01$) ($p < 0.001$ for trend) for control, matched control and the 19, 38, 81 and 160 mg/kg-bw groups, respectively.[2]

Kidney tissue from this investigation (Jorgenson *et al.*, 1985) has recently been microscopically reevaluated for evidence of cytotoxicity and regeneration (Hard and Wolf, 1999; Hard *et al.*, in press). Detailed results of this reevaluation are presented in Table 7. This reexamination included a portion of animals in the untreated control group and all animals in the four dose groups sacrificed at 104 weeks (the number of tissues in each group for which it was possible to evaluate chloroform-related cytotoxicity ranged from 16 to 48). Kidneys from rats at interim time points were also examined; however, slides from water-matched controls and the low-dose group at 2 years were not available. Slides from the 104-week sacrifices were read independently by each of three authors; those for interim time points for selected dose groups were evaluated in blinded fashion by one of the authors.

Toxic injury in proximal tubular epithelial cells was observed in all high-dose (1800 mg/L, the dose at which there was a statistically significant increase in tumour incidence) males at all time points and approximately half of animals receiving the second highest dose (900 mg/L) for 18 or 24 months. None of the other treatment groups or controls had these characteristic changes. The chloroform-associated alterations were characterized by slightly increased basophilia, cytoplasmic vacuolation, karyomegaly, anisokaryosis, nuclear crowding and mild tubular hyperplasia. The cytotoxic tubular lesions, occasional foci of atypical tubular hyperplasia and incipient renal tubular tumours were all located in the mid to deep cortex.

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[2] These incidences were verified by U.S. EPA (1996a) in a reexamination of the records of the data for individual animals.

Although a systematic evaluation was not possible due to degradation of the slides and frequent autolytic change, the authors confirmed that such changes were also present in males of the same strain in the National Cancer Institute (NCI) bioassay in which exposure was by corn oil gavage (NCI, 1976). An incidental finding was the striking difference in the dimensions of renal tumours induced by chloroform administered by corn oil gavage (approximately 2-fold greater) compared with those arising from exposure via drinking water in the investigation by Jorgenson *et al*. (1985) (Hard *et al*., in press).

In a recent study reported currently only as an abstract, Gollapudi *et al.* (1999) exposed transgenic p53$^{+/-}$ mice (who respond most effectively to mutagenic carcinogens) to chloroform by gavage in corn oil at doses up to 140 mg/kg-bw per day (males) or 240 mg/kg-bw per day (females) for up to 26 weeks. Wild-type mice were also included in the protocol. Although renal tubular regeneration and proliferation of renal tubular epithelial cells were observed in males, there were no treatment-related increases in the incidence of any tumours.

2.4.3.4.3 Nose

In a chronic study (Yamamoto, 1996), F344 rats were exposed to 0, 10, 30 or 90 ppm (0, 49, 147 or 441 mg/m^3) and BDF1 mice were exposed to 0, 5, 30 or 90 ppm (0, 25, 147 or 441 mg/m^3) for 104 weeks. It was reported that ossification was induced at 10 ppm (49 mg/m^3) in rats and at 5 ppm (25 mg/m^3) in mice. Other observations included ossification of nasal turbinates (rats) or nasal septum (mice), necrosis and respiratory metaplasia of the olfactory epithelium and goblet cell hyperplasia in the respiratory epithelium in both sexes of rats and mice, although it was not specified at which concentrations these effects were observed.

In spite of the overt toxicity and increased cell proliferation in these epithelial tissues in the nose, no tumours have been noted in this tissue in any of the chronic toxicity studies, including the inhalation study in which nasal tissues appear to have been very carefully evaluated (Yamamoto, 1996).

2.4.3.5 Genotoxicity

In recent assessments by both an IPCS Task Group (WHO, 1994) and an ILSI Expert Panel (ILSI, 1997), it has been concluded that the weight of evidence of the genotoxicity of chloroform is negative.

Conclusions of the IPCS Task Group in this regard were as follows (WHO, 1994):

> The weight of the available evidence indicates that chloroform has little, if any, capability to induce gene mutation, chromosomal damage and DNA repair. There is some evidence of low-level binding to DNA, however. Chloroform does not appear capable of inducing unscheduled DNA synthesis *in vivo*.

The ILSI Expert Panel (ILSI, 1997) concluded:

For the assessment of available data on genotoxicity of chloroform, the Expert Panel adopted the comprehensive, quantitative weight of evidence approach to evaluate large, heterogeneous genetic toxicology databases which are not amenable to test-by-test critiques, published by the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC) (Lohman *et al.*, 1992). The scores developed by the ICPEMC weight of evidence program are relative DNA reactivity scores. The theoretical maximum positive score is +100 and the maximum negative score is –100. For over 100 chemicals evaluated and classified on this basis, the highest positive score obtained was 49.7 (triazaquone) and the lowest negative score was –27.7 (ethanol). Chloroform results from over 40 studies yield a quantitative net negative score (–14.33) in this system, indicating that the weight of evidence supports a non-genotoxic classification (Brusick *et al.*, 1992; Lohman *et al.*, 1992).

An overview of identified studies on the genotoxicity of chloroform that have been reviewed independently for this evaluation is provided in Table 8, subdivided by those considered to be "core" or other. For the core tests, *in vitro*, chloroform has been negative in the vast majority of assays in *Salmonella typhimurium* and *Escherichia coli.* However, there is one report of it being positive/weakly positive in four strains of *Salmonella* (Varma *et al.*, 1988) and weakly positive in one strain of *Salmonella* (Pegram *et al.*, 1997). There are no relevant *in vitro* core cytogenetic results; although results have been mixed in indicator tests primarily for sister chromatid exchange (SCE), consistently negative results were reported for unscheduled DNA synthesis (UDS) in a wide range of animal and human cells. *In vivo*, there are three negative and one equivocal mouse micronuclei studies. There have also been one positive micronuclei assay in rat kidney (Robbiano *et al.*, 1998), one positive micronuclei assay in rat liver (Sasaki et al., 1998), one positive assay for chromosomal aberrations in bone marrow in the rat (Fujie *et al.*, 1990) and one weakly positive investigation of chromosomal aberrations in hamster bone marrow (Hoechst, 1987). For other endpoints, *in vivo*, results have been consistently negative, with the exception of the weak DNA binding reported by Colacci *et al.* (1991) and Pereira *et al.* (1982), mixed results for sperm abnormalities and a positive SCE in mouse bone marrow (Morimoto and Koizumi, 1983).

While, overall, the weight of evidence of the genotoxicity of chloroform is negative, the possibility of a weak positive response in rats cannot be excluded on the basis of the following observations:

1. One of the only positive (although marginal) results in the *Salmonella* gene mutation assay *in vitro* was observed in a TA1535 strain transfected with the rat glutathione-S-transferase T1-1 gene (Pegram *et al.*, 1997).

2. There was at least some activity in all of the identified *in vivo* studies in rats, as follows:

    - A 3.3-fold increase in micronuclei induction in rat kidney cells (4.42 vs. 1.33 at 4 mmol/kg) (Robbiano *et al.*, 1998),
    - An 8.75-fold (intraperitoneal) and 6-fold (per os) increase in chromosomal breaks in rat bone marrow (Fujie *et al.*, 1990), and
    - Weak (3-fold) induction of micronuclei in liver of partially hepatectomized rats at 400 mg/kg-bw (Sasaki *et al.*, 1998).

While each of these results was acquired in non-standard tests and can be considered questionable in its own right, their collective interpretation gives rise to uncertainty about conclusions concerning the weight of evidence of the genotoxicity of chloroform; further investigation of the nature of the induction of these effects (i.e., whether direct or secondary) is desirable.

Data on the genotoxicity of the metabolites of chloroform are limited. For example, investigations of the genotoxic potential of phosgene, the highly reactive oxidative metabolite of chloroform, have not been identified (ILSI, 1997). There are some data relevant to the assessment of the genotoxicity of reductive metabolites of chloroform. As indicated above, in an assay in which copies of the rat glutathione transferase gene were engineered into *Salmonella*, while bromodichloromethane produced mutagenic conjugates, the effects of chloroform were only marginal (less than a doubling at concentrations greater than 10 000 ppm) (Pegram *et al.*, 1997). Additional supportive evidence that direct interaction of reductive metabolites of chloroform with DNA is unlikely is provided by another of the chlorinated methanes, namely carbon tetrachloride. While this compound is metabolized almost exclusively by reductive pathways to free radicals that cause severe liver toxicity, the preponderance of data indicates that it is not mutagenic (Morita *et al.*, 1997).

2.4.3.6 Reproductive and developmental toxicity

Reproductive and developmental assays involving oral exposure in mice, rats and rabbits were identified. In no assay were teratogenic effects reported, and effects on reproduction were observed only at dose levels that were maternally toxic. In a continuous breeding protocol with CD-1 mice, there were no effects on fertility or reproduction in the F1 generation administered 41 mg/kg-bw per day by gavage (in corn oil); at this dose, there was hepatocellular degeneration in females (LOAEL) (EHRT, 1988). A decrease in fetal body weight but no teratogenic effects were reported in Sprague-Dawley rats intubated (in corn oil) with 126 mg/kg-bw on days 6–15 of gestation (maternal LOEL: 50 mg/kg-bw per day) (Thompson *et al.*, 1974). In a similar protocol with higher dose levels, Ruddick *et al.* (1983) reported decreased fetal body weight in Sprague-Dawley rats at 400 mg/kg-bw (maternal LOAEL: 100 mg/kg-bw per day). No dose-related reproductive or developmental changes were observed in rabbits administered chloroform by stomach tube (in corn oil) on days 6–18 of gestation (maternal LOEL: 50 mg/kg-bw per day) (Thompson *et al.*, 1974).

Results across the few inhalation bioassays identified were consistent. In Sprague-Dawley rats exposed to 0, 30, 100 or 300 ppm (0, 147, 490 or 1470 mg/m^3), there was a significant decrease in body weight at the lowest concentration (maternal LOEL) (Schwetz *et al.*, 1974). Hoechst (1988) exposed Wistar rats to identical dose levels and reported decreased food consumption and body weight gain at 30 ppm (147 mg/m^3) (maternal LOAEL). At 30 ppm (147 mg/m^3) in both studies, fetal crown–rump length was significantly reduced, although not in a dose-related manner in the former study. Although adverse skeletal and visceral effects were reported by Schwetz *et al.* (1974), they were not dose-related; no teratogenic effects were observed by Hoechst (1988). Hoechst (1990, 1993) repeated the bioassay at lower concentrations and reported that maternal body weight was reduced at 10 ppm (49 mg/m^3) (maternal LOAEL), although there were no effects upon reproductive or developmental parameters at this concentration.

2.4.3.7 Toxicokinetics and mode of action

There is considerable information available concerning the potential mode of induction of tumours by chloroform. This includes metabolic studies and a vast array of investigations of proliferative response in target organs following exposure via regimens similar to those in cancer bioassays where increases in tumours have been observed. These data have been generated to investigate primarily the hypothesized mode of action for tumour induction in rodents that requisite precursor steps are metabolism of chloroform by the target cell population, induction of sustained cytotoxicity by oxidative metabolites and subsequent regenerative cell proliferation.

2.4.3.7.1 Kinetics and metabolism and relationship with tissue damage

Both oxidative and reductive pathways of chloroform metabolism have been identified, although data *in vivo* are limited. The oxidative pathways generate reactive metabolites including, perhaps exclusively, phosgene (Pohl *et al.*, 1977; Pohl and Krishna, 1978) (determined *in vitro*, with phenobarbital induction), while the reductive pathway generates the dichloromethylcarbene free radical (Wolf *et al.*, 1977; Tomasi *et al.*, 1985; Testai and Vittozzi, 1986) (determined *in vitro* and *in vivo*, both with and without phenobarbital induction). The metabolism of chloroform proceeds through a P450-dependent activation step, regardless of whether oxidative or reductive reactions are occurring. The balance between oxidative and reductive pathways depends on species, tissue, dose and oxygen tension.

Phosgene is produced by oxidative dechlorination of chloroform to trichloromethanol, which spontaneously dehydrochlorinates (Mansuy *et al.*, 1977; Pohl *et al.*, 1977).

The electrophilic metabolite phosgene binds covalently to nucleophilic components of tissue proteins (Pohl *et al.*, 1980). It also interacts with other cellular nucleophiles (Uehleke and Werner, 1975) and binds to some extent to the polar heads of phospholipids (Vittozzi *et al.*, 1991). Alternatively, phosgene reacts with water to release carbon dioxide and hydrochloric acid (Fry *et al.*, 1972; D.M. Brown *et al.*, 1974). The interaction of phosgene with glutathione results in the formation of S-chlorocarbonyl glutathione, which can either interact with an additional glutathione to form diglutathionyl dithiocarbonate (Pohl *et al.*, 1981) or form glutathione disulphide and carbon monoxide (Ahmed *et al.*, 1977; Anders *et al.*, 1978). Incubation of mouse renal microsomes with glutathione increases production of these metabolites from chloroform and decreases irreversible binding to proteins and further metabolism to carbon dioxide (Smith and Hook, 1984). Reduced glutathione is capable of scavenging essentially all chloroform metabolites produced in incubations with mouse liver microsomes when chloroform concentrations are not too high (Vittozzi *et al.*, 1991).

Carbon dioxide is the major metabolite of chloroform generated by the oxidative pathway *in vivo*.

The relative importance of the minor pathways of phosgene metabolism depends upon the availability of glutathione, other thiols and other nucleophilic compounds, such as histidine and cysteine.

Dehydrochlorination of trichloromethanol produces 1 mol of hydrochloric acid, and hydrolysis of phosgene produces 2 more, so that 3 mol of hydrochloric acid are produced in the conversion of chloroform to carbon dioxide. Both products of oxidative activation, phosgene and hydrochloric acid, can cause tissue damage. Phosgene, as noted above, can bind covalently to cellular nucleophiles. Local acidification consequent to hydrochloric acid generation may also be cytotoxic.

Available data indicate that the toxicity of chloroform is attributable to metabolites. In the liver, for example, both the incidence and severity of toxicity correlate with the level of covalent binding of chloroform metabolites to tissue macromolecules, and phosgene is believed to be quantitatively responsible for the irreversible binding of chloroform metabolites to liver components (Pohl *et al*., 1980). The extent of chloroform-induced hepatic necrosis also correlates with the extent of covalent binding to protein in male and female rats and in male mice (Ilett *et al*., 1973; B.R. Brown *et al*., 1974). This covalent binding is more prevalent within the areas of necrosis (Ilett *et al*., 1973; Tyson *et al*., 1983), and the association of metabolism with toxicity is further supported by localization of binding to necrotic lesions (Ilett *et al*., 1973). The results of *in vitro* studies are consistent, in that irreversible binding to macromolecules in rat and human liver microsomes requires prior metabolism (Cresteil *et al*., 1979).

Increased covalent binding of chloroform metabolites in the liver also occurs when glutathione is depleted, while some degree of protection is conferred if glutathione or a precursor is administered (Stevens and Anders, 1981). Since covalent binding of a chloroform metabolite with glutathione precedes and becomes maximal prior to the chloroform-induced hepatic cytotoxicity, depletion of glutathione may contribute to the observed cytotoxicity as it does to covalent binding (Stevens and Anders, 1981).

In mice, covalent binding of chloroform to renal proteins and microsomes is correlated with the degree of renal tubular necrosis (Ilett *et al*., 1973; Smith and Hook, 1983, 1984). Strain- and sex-related differences in sensitivity of mice to nephrotoxicity are also correlated with the ability of the kidney to metabolize chloroform (Taylor *et al*., 1974; Clemens *et al*., 1979; Pohl *et al*., 1984; Smith *et al*., 1984; Mohla *et al*., 1988; Henderson *et al*., 1989; Hong *et al*., 1989). In an investigation in F344 rats, however, it was concluded that intrarenal bioactivation of chloroform by cytochrome P450 did not appear to play a major role in nephrotoxicity (Smith *et al*., 1985).

The toxicity of chloroform has, therefore, traditionally been attributed principally to the electrophilic metabolite phosgene. However, Vittozzi and coworkers (Testai *et al*., 1990; Vittozzi *et al*., 1991) have argued that reductive activation of haloalkanes in physiologically hypoxic tissues should be given greater consideration. Physiologically hypoxic tissues include the centrilobular region of the liver, where haloalkane hepatotoxicity is largely localized. Physiological partial pressures of oxygen in the liver range from 0.13 to 8 kPa (1 to 60 mmHg), with a mean around 2.7 kPa (20 mmHg), with the lowest values located in the centrilobular region (de Groot and Noll, 1989). Although the dichloromethylcarbene radical could account for many of the reactive properties of chloroform, a large amount of circumstantial evidence argues against the significance of the anaerobic pathway of chloroform metabolism under normal conditions. The anaerobic pathway is observable only in phenobarbital-induced (or naphthoflavone-induced) animals or in tissues prepared from them; microsomes from uninduced animals display negligible reducing activity (Testai and Vittozzi, 1986). Chloroform is relatively ineffective compared with other haloalkanes as a source of free radicals or binding to P450 enzymes, even under the most favourable of test conditions (de Groot and Noll, 1989). Large species differences also exist in the ability of liver microsomes to catalyse reductive activation of chloroform, with microsomes derived from rats and humans being among the least active in this regard (Butler, 1961; Vittozzi *et al.*, 1991) and microsome preparations from mice being only slightly active (Butler, 1961).

Recently, direct experimental evidence has linked oxidative metabolism with tissue toxicity. The reactive intermediates generated by the oxidative and reductive pathways of chloroform metabolism bind to phospholipids differently. Oxidative products bind to the polar heads of the phospholipid molecule, while reductive metabolites bind to the fatty acid tails (De Biasi *et al.*, 1992). This feature has been used experimentally *in vitro* to distinguish covalent binding resulting from chloroform oxidation from that resulting from chloroform reduction. Ade *et al.* (1994) investigated the amount of chloroform binding to proteins and to the polar heads and fatty acid portions of phospholipids in microsomes prepared from kidneys of uninduced DBA/2J mice. Protein and lipid binding were correlated with hormonal status (males, females and testosterone-treated females) only under aerobic conditions, indicating that oxidative metabolism is involved in the gender-specific renal toxicity of chloroform. Combining data from previous (Testai *et al.*, 1990; De Biasi *et al.*, 1992) and current studies, Ade *et al.* (1994) also demonstrated a direct linear correlation between adducts to polar phospholipid heads and adducts to protein in microsomal preparations from livers of B6C3F1 mice and kidneys of DBA/2J mice, even under different experimental conditions. Of the total binding to microsomal phospholipids at 20% oxygen partial pressure, less than 25% was to the fatty acid tails (and presumably derived from reductive processes). Supplementation of an incubation medium with 3 mM glutathione under room air completely abolished binding to liver microsomal lipids (Testai *et al.*, 1990, 1992), but a small amount of residual binding to kidney microsomal lipids persisted under these conditions (Ade *et al.*, 1994).

Convincing evidence of the role of oxidative metabolism in the toxicity of chloroform has also been acquired recently in male B6C3F1, Sv/129 wild-type and Sv/129 CYP2E1 null mice exposed by inhalation for 6 hours per day for 4 days. B6C3F1 and Sv/129 wild-type mice exposed to chloroform alone had extensive hepatic and renal necrosis with significant regenerative cell proliferation and minimal toxicity in the nasal turbinates with focal periosteal proliferation (Constan *et al.*, 1999). These effects were not observed in mice pretreated with a P450 inhibitor (1-aminobenzotriazole). No adverse effects were observed in the Sv/129 CYP2E1 null mice.

These observations strongly support the conclusion that under normal conditions, reductive metabolism of chloroform in liver and kidney is minor and reductive dechlorination is not a quantitatively significant pathway in the human bioactivation of chloroform. However, because the reductively generated metabolites of chloroform are not effectively scavenged by glutathione, they may have contributed to the marked lipid peroxidation observed at high substrate concentrations and low oxygen tensions in experimental studies *in vitro* (Testai *et al.*, 1990, 1992; Ade *et al.*, 1994).

The primary, if not only, enzyme catalysing metabolism at low concentrations of chloroform is cytochrome P4502E1 (CYP2E1) (Brady *et al.*, 1989; Guengerich *et al.*, 1991). CYP2E1, induced by ethanol, n-hexane, secondary ketones, isopropanol and imidazole, is active in the metabolism of a wide variety of low-molecular-weight compounds in addition to the haloalkanes. The dominant involvement of CYP2E1 is confirmed by studies with chemical inducers of this isozyme, which lead to marked increases in metabolism of chloroform in microsomes from treated rats (Brady *et al.*, 1989). In contrast, treatment with phenobarbital, which reduces the amount of CYP2E1 (Nakajima *et al.*, 1995a,b), inhibits metabolism of chloroform (Brady *et al.*, 1989). In addition, Brady and his coworkers have demonstrated competitive substrate inhibition by CYP2E1 antibodies in rat liver microsomes, indicating that CYP2E1 is responsible for at least 80% of the microsomal metabolism of chloroform at lower doses.

In earlier studies, reviewed in Pohl (1979), it had been demonstrated that inducers of the CYP2B family could also increase the conversion of chloroform to carbon dioxide. Since it is presumed that metabolism through the pathway that generates carbon dioxide results in covalent binding to tissue components, these studies indicated that the CYP2B pathway may also generate reactive intermediates. Nakajima *et al.* (1991, 1995a,b) proposed that CYP2E1 is a lower-K_m enzyme that is entirely responsible for metabolism of chloroform at low chloroform concentrations, while CYP2B1/2 is a high-K_m isozyme whose activity is demonstrable only at high chloroform concentrations. Studies with purified reconstituted enzyme systems also indicate that CYP2E1 is active and CYP2B1 inactive in the metabolism of chloroform at low concentrations of substrate (Brady *et al.*, 1989). Although not optimized to demonstrate comparison between *in vitro* and *in vivo* determinations, Mohla *et al.* (1988) estimated the K_m values for the two isozymes isolated from the kidneys of BALB/c mice to be 0.6 ± 0.2 mM (CYP2E1) and 20.2 ± 6.8 mM (CYP2B1). The results of recent studies in SV/129 CYP2E1 null mice (Constan *et al.*, 1999) indicate, however, that the role of CYP2B is minimal, even at high doses.

Regional distribution of lesions in the liver of rats and mice also correlates well with the hepatic distribution of CYP2E1 and glutathione. The highest concentrations of CYP2E1 in both uninduced and induced rat and human liver are present in the centrilobular region (Ingelman-Sundberg *et al.*, 1988; Tsutsumi *et al.*, 1989; Johansson *et al.*, 1990; Dicker *et al.*, 1991). In comparison, concentrations of the phosgene-scavenging agent glutathione in the centrilobular region are only about half those in the periportal region (Smith *et al.*, 1979).

In rats administered ^{14}C-chloroform to identify those tissues with chloroform-metabolizing capability by autoradiography, the generation of carbon dioxide and incorporation of ^{14}C into macromolecules *in vitro* were subsequently investigated (Löfberg and Tjälve, 1986). There were correlations between the ability of tissues to metabolize chloroform *in vivo* and *in vitro* and between sites accumulating metabolites *in vivo* and *in vitro*. Because radiolabel was accumulated in trichloroacetic acid-insoluble material, it was assumed to represent covalently bound metabolite. Tissues with chloroform-metabolizing ability included liver; kidney cortex; tracheal, bronchial, olfactory and respiratory nasal mucosa; and esophageal, laryngeal, tongue, gingival, cheek, nasopharyngeal, pharyngeal and soft palate mucosa. Of these, the liver was the most active, followed by the nose and kidney. Values for liver, kidney and nose (in dpm/mg wet tissue) are presented below:

	Tissue-bound ^{14}C	**$^{14}CO_2$**
Liver	48.0	143.5
Nasal olfactory mucosa	22.3	51.0
Nasal respiratory mucosa	17.5	45.2
Kidney cortex	10.5	38.8

2.4.3.7.2 Physiologically based pharmacokinetic (PBPK) models

Corley *et al.* (1990) developed the first extensive model for chloroform. Liver and kidney were described individually and were sites of metabolism for chloroform. The maximum velocity of metabolism in the kidney was scaled to the maximum velocity in the liver based on relative tissue volumes and a proportionality constant. In order to fit gas uptake data, terms were added to allow for loss and resynthesis of metabolizing enzyme. Reitz *et al.* (1990) modified the Corley model to include description of a pharmacodynamic endpoint, the induction of cytotoxicity in the liver. Two dose surrogates were considered: average daily macromolecular binding and cytotoxicity. The latter was chosen as the dose surrogate best reflecting carcinogenicity. Gearhart *et al.* (1993) modified tissue to blood partition coefficients and metabolism according to body temperature and were able to fit gas uptake data without the need to describe enzyme loss and resynthesis. Borghoff and coworkers (Dix *et al.*, 1994; Dix and Borghoff, 1995) incorporated absorption from the stomach as well as the intestinal tract and also accounted for gastric emptying time. In 1996, Lilly developed a model for bromodichloromethane that featured subdivision of the liver and kidney compartments into regions of high and low metabolic activity. The combination of this approach with the two-compartment absorption model of Borghoff and coworkers resulted in the most recent PBPK model for chloroform in animals (ILSI, 1997).

For this assessment, the "hybrid" animal model of the ILSI Expert Panel (ILSI, 1997) was revised and extended to humans and modified to permit accommodation of multimedia exposures (ICF Kaiser, 1999).

For the present assessment, several variants of the ILSI (1997) model in rats were developed.[3] In all, the $V_{max}KC$ for the kidney adopted in the ILSI (1997) model was revised, based on the equation and appropriate values reported by Corley *et al.* (1990). The resulting value was 0.094 (proportionality constant A = (V/Skidney)/(V/Sliver). $V_{max}KC = (A*VKC*V_{max}LC)/VLC = 0.094$).

In one variant, physiological and anatomical parameters for the ILSI model were updated based on more recent data reported by Brown *et al.* (1997). In addition, the ILSI model considered water consumption in rats as a 12 hours on, 12 hours off cycle, whereas one variant of the model developed for this assessment also considered and incorporated actual water consumption patterns by male rats over a 24-hour period (Yuan, 1993; ICF Kaiser, 1999). Physiological and metabolic parameters for the various variants are presented in Table 9.

For the present assessment, a model was also developed for the dog. The physiological and anatomical parameters were taken from Brown *et al.* (1997), while metabolic parameters were based on the average of rat and human parameters reported by Corley *et al.* (1990). The fractional subvolumes for the liver were assumed to be the same as those reported for the rat by ILSI (1997), which were estimated by quantitative evaluation of immunohistochemically stained slides of liver lobule reported by Tsutsumi *et al.* (1989) and Buhler *et al.* (1992).

For the human model, the physiological and anatomical parameters were also derived from Brown *et al.* (1997) with the exception of the ventilation rate and cardiac output, which were related to an assumed breathing rate of 23 m^3/day (Health Canada, 1994). The partition coefficients and rate constants in ILSI (1997) were maintained. Liver tissue subvolumes were assumed to be the same as in the rat, based on Tsutsumi *et al.* (1989) and Buhler *et al.* (1992), while kidney was subdivided into a 70:30 cortex:non-cortex ratio as described by ICRP (1992). Human metabolic parameters were taken from Corley *et al.* (1990); these had been determined *in vitro* in eight human liver samples. Kidney rate constants were based on the relationship of activity observed in the microsomal fraction of kidneys to the activity observed in the microsomal fraction of the liver based on *in vitro* results reported by Corley *et al.* (1990) but supported by data on metabolism of two known substrates of CYP2E1 by microsomal fractions of the kidney and liver from 18 humans (Amet *et al.*, 1997).

Results from the human model were compared with data on total metabolized parent and exhaled chloroform reported by Fry *et al.* (1972) in an investigation in which chloroform was administered to male and female volunteers in olive oil or gelatin capsules. Exhaled chloroform was measured for up to 8 hours following exposure, and the total percentage of the dose exhaled unchanged was calculated by extrapolation to infinite time. Human model simulations conducted using a single-compartment description of oral uptake were closer to the observations of Fry *et al.* (1972) than those estimated using a multi-compartment description. Therefore, while a multi-compartment description was necessary in the rat model, a single-compartment description of oral uptake was used in estimating human equivalent concentrations.

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[3] Body weight was study-specific average body weight from the Jorgenson *et al.* (1985) study.

The model was also modified to permit assessment of exposure to chloroform from all likely sources, including air, water and food. The exposure scenario was modelled within a 24-hour day and included inhalation, ingestion and dermal absorption from one 10-minute shower, a brief washing-up period before retiring, discrete periods of food and water consumption and inhalation of chloroform at various concentrations (ICF Kaiser, 1999).

2.4.3.7.3 Investigations of regenerative cell proliferation

The vast array of investigations of proliferative response in target organs in which tumours have been observed are reviewed here. Studies on cell proliferation have been conducted in BDF1 and B6C3F1 mice and F344 and Osborne-Mendel rats, by gavage, drinking water or inhalation, for exposure periods ranging from 1 day up to 22 weeks, with cell proliferation being quantified by bromodeoxyuridine immunohistochemistry. A brief summary of the results of these studies is presented here. Additional data on exposure–response for these proliferative lesions are included in the supporting documentation for this assessment (Health Canada, 1999).

Liver

(a) Oral exposure

Compensatory hyperplasia is considered an important component of the response of the liver to necrosis. Hepatic cell turnover, as monitored by hepatic labelling index, is often determined as a surrogate measure, therefore, for hepatic cytotoxicity. Increases in hepatic labelling index may be sustained at the initial levels, but more often are transient and likely represent regenerative growth in response to cell death produced by repeated exposures to chloroform.

Chloroform administered to female B6C3F1 mice by corn oil gavage for 4 days increased the hepatic labelling index at 238 and 477 mg/kg-bw per day but not at 90 mg/kg-bw per day. The hepatic labelling index was still elevated at 3 weeks relative to controls but was decreased relative to the hepatic index observed at the 4-day time point (Larson *et al.*, 1994a). These results are consistent with the observation of liver tumours in females of this strain following administration of similar doses (238 and 477 mg/kg-bw per day) for 78 weeks (NCI, 1976). Melnick *et al.* (1998) exposed female B6C3F1 mice by gavage in corn oil for 3 weeks and reported a significant increase in hepatic labelling index at 110, 238 and 477 mg/kg-bw per day (top two doses similar to those administered to females in the NCI bioassay); their protocol did not include a dose level of 90 mg/kg-bw per day. In male B6C3F1 mice administered chloroform by corn oil gavage (Larson *et al.*, 1994c), the hepatic labelling index was increased relative to controls in the 34–277 mg/kg-bw per day dose range after 4 days of administration. After 3 weeks of administration, the hepatic labelling index was not elevated compared with controls at 34 and 90 mg chloroform/kg-bw per day but was still significantly elevated at 138 and 277 mg/kg-bw per day. At 138 mg/kg-bw per day, the increase in proliferation was less than at 4 days; at 277 mg/kg-bw per day, it was similar to that observed at 4 days (Larson *et al.*, 1994c). These results (i.e., sustained increases in proliferative response at similar doses) are consistent with increases in hepatic tumour incidence observed in the NCI bioassay in males at 138 and 277 mg/kg-bw per day.

Pereira (1994) examined the hepatic labelling index in female B6C3F1 mice over longer periods (263 mg chloroform/kg-bw by corn oil gavage for 159 days). Initially (at 5 days), marked regenerative hyperplasia was observed. The hepatic labelling index remained elevated but declined, relative to the increase observed at 5 days, steadily to the last observation point (159 days of administration). However, the increase in labelling index over respective controls was relatively constant over the period of administration, ranging from 5 times the control value at 5 days to 7 times at 159 days.

Exposures of female B6C3F1 mice to chloroform in drinking water at concentrations similar to those used in the Jorgenson *et al.* (1985) bioassay (60–1800 mg/L) did not increase the hepatic labelling index after 4 days or 3 weeks of administration (Larson *et al.*, 1994a).[4] This is consistent with the lack of increase in hepatic tumour incidence observed in this strain in this cancer bioassay and contrasts with the results described above in which similar daily doses administered by gavage increased cellular proliferation and tumours.

In male F344 rats administered chloroform by corn oil gavage, an increase in hepatic labelling index was observed following 4 days of exposure to 90 and 180 mg chloroform/kg-bw and following 3 weeks of administration of 180 but not 90 mg/kg-bw (Larson *et al.*, 1995b). However, the degree of elevation relative to controls was less after 3 weeks (4.5 times control) of administration than after 4 days (17 times control). These doses were similar to those administered to male Osborne-Mendel rats in the NCI bioassay in which tumours were observed at 180 mg/kg-bw per day. In female F344 rats administered chloroform by corn oil gavage, the hepatic labelling index was increased following 4 days or 3 weeks of administration at doses between 100 and 400 mg/kg-bw per day (Larson *et al.*, 1995a). This increased hepatic labelling index was sustained through 3 weeks of exposure at the 200 and 400 mg/kg-bw doses. While it declined relative to the increase observed at 4 days of administration of these doses, there was still an approximate 3-fold increase in relation to controls between 4 days and 3 weeks.

An increase in hepatic labelling index was not observed in male F344 rats administered chloroform at concentrations up to 1800 mg/L in drinking water for either 4 days or 3 weeks (Larson *et al.*, 1995b), consistent with the lack of increase in liver tumours observed in Osborne-Mendel rats exposed to similar concentrations in the cancer bioassay of Jorgenson *et al.* (1985).

(b) Inhalation exposure

Larson *et al.* (1996) exposed B6C3F1 mice to chloroform for 6 hours per day for up to 13 weeks, in dosing regimens with varying days per week exposure. An increase in hepatocyte labelling index was observed at 30 ppm (147 mg/m^3) in females exposed on a daily basis for 3 and 6 weeks, but not at 13 weeks. Increases at 90 ppm (441 mg/m^3) were observed in both sexes at most durations of exposure, where exposure was for 7 days per week. However, exposure for 5 days per week was carried out only in a 13-week protocol. Butterworth *et al.* (1998) exposed female B6C3F1 (lacI transgenic) mice on a daily basis, 6 hours per day, for up to 180 days and reported no increase in hepatocyte labelling index at 10 ppm (49 mg/m^3), a "borderline" response at 30 ppm (147 mg/m^3) and "substantial regenerative cell proliferation" at 90 ppm (441 mg/m^3) at all time points. Templin *et al.* (1998) exposed BDF1 mice for 5 days per week for up to 13 weeks and reported regenerative proliferation in both sexes only at 90 ppm (441 mg/m^3), the highest concentration. At 13 weeks, the increase was observed only in females. At this concentration, in the only identified inhalation carcinogenesis bioassay that was conducted in the same strain of mice, borderline increases in hepatic tumour incidence were reported (Yamamoto, 1996). These mice were exposed for 6 hours per day, 5 days per week.

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[4] Water consumption decreased during the first week of exposure but recovered to near control levels for the remainder of the study. For the 4-day exposure study, concentrations of 0, 60, 200, 400, 900 and 1800 mg/L resulted in average daily doses of 0, 16, 26, 54, 81 and 105 mg/kg-bw per day. For the 3-week study, the corresponding average daily doses were 0, 16, 43, 83, 184 and 329 mg/kg-bw per day (Larson *et al.*, 1994a).

Templin *et al.* (1996b) exposed male and female F344 rats to various regimens for up to 13 weeks. Exposure was daily, with the exception of the 13-week protocol, in which exposures were for either 5 or 7 days per week. Increased labelling index in both sexes was observed only at the highest chloroform concentration of 300 ppm (1470 mg/m^3), for exposures of both 5 and 7 days per week. However, no increases in hepatic tumour incidence were reported in this strain exposed to up to 90 ppm (441 mg/m^3) in the only identified inhalation carcinogenesis bioassay (Yamamoto, 1996), in which exposure was for 5 days per week.

Kidney

Increased kidney cell proliferation has been observed mainly in the proximal convoluted tubules of the cortex, extending into the straight proximal tubules of the outer stripe of outer medulla at higher doses.

(a) Oral exposure

In male B6C3F1 mice, doses of 0, 34, 90, 138 and 277 mg chloroform/kg-bw per day by gavage induced a dose-dependent increase in labelling index of proximal tubules at all doses after 4 days (Larson *et al.*, 1994c). At 3 weeks, there was a diminution in the response at all dose levels, and only at doses of 138 and 277 mg/kg-bw per day were the labelling indices significantly elevated over controls (Larson *et al.*, 1994c). There were no increases in renal tumour incidence in the cancer bioassay in which male mice of this strain were exposed to 138 and 277 mg/kg-bw per day in corn oil for 78 weeks (NCI, 1976). After administration of chloroform in drinking water to female B6C3F1 mice for 3 weeks, the labelling index in the medulla was significantly increased at 200 mg/L (Larson *et al.*, 1994a). This was the same as the lowest dose in the Jorgenson *et al.* (1985) study, in which renal tumours were not observed in this strain following administration in drinking water of concentrations up to 1800 mg/L.

Gollapudi *et al.* (1999) reported proliferation of renal tubular epithelial cells in male transgenic p53$^{+/-}$ mice exposed to 140 mg/kg-bw per day by gavage in corn oil for 13 weeks.

Although a carcinogenesis bioassay in F344 rats exposed via ingestion is not available as a basis for comparison, dose-dependent increases in renal cell proliferation have also been demonstrated in this strain following administration by corn oil gavage (Larson *et al.*, 1995a,b). In the male F344 rat, the labelling index in the renal cortex was increased only at the highest dose (180 mg/kg-bw per day) after 4 days of administration in corn oil. In the parallel studies in female F344 rats, dose-dependent increases in the labelling index in the renal cortex were observed at doses of 100 mg/kg-bw per day and above administered by corn oil gavage at both 4 days and 3 weeks (Larson *et al.*, 1995a).

Templin *et al.* (1996a) compared male F344 and Osborne-Mendel strains after a single gavage exposure at an observation point of 2 days. Although the authors concluded that these strains were about equally susceptible to chloroform-induced renal injury, a statistically significant increase in labelling index was observed at a much lower dose (10 mg/kg-bw) in the kidney of the Osborne-Mendel rat than in the F344 rat (90 mg/kg-bw) (Templin *et al.*, 1996a). This is the only published investigation of chloroform-associated renal cell proliferation as measured by labelling index in the Osborne-Mendel rat, a function primarily of the lack of commercial availability of pathogen-free colonies of this strain.

Chloroform administered to male F344 rats at concentrations in drinking water similar to those in the cancer bioassay of Jorgenson *et al.* (1985) produced no increase in renal cell labelling index at either 4 days or 3 weeks (Larson *et al.*, 1995b). This attests to the greater sensitivity of male Osborne-Mendel rats, in which renal tumours in males were observed at the highest dose in the study by Jorgenson *et al.* (1985).

(b) Inhalation exposure

In a 13-week study in which chloroform was administered to B6C3F1 mice at doses ranging from 0.3 up to 90 ppm (1.5 to 441 mg/m^3) by inhalation (Larson *et al.*, 1996), the renal labelling index in male mice was significantly increased at concentrations of 30 and 90 ppm (147 and 441 mg/m^3) when exposure was for 7 days per week and at 10 ppm (49 mg/m^3) when exposure was for 5 days per week. It was not increased in female mice at any concentration. Similar results were reported by Templin *et al.* (1998) in an assay in BDF1 mice, with daily exposure. An inhalation cancer bioassay has not been conducted with either of these strains.

A 7- to 10-fold increase in labelling index over controls was observed in the kidneys of male, but not female, BDF1 mice exposed to 30 and 90 ppm (147 and 441 mg/m^3) (but not 5 ppm [25 mg/m^3]) chloroform by inhalation, 6 hours per day for 4 consecutive days (Templin *et al.*, 1996c). This observation supports the contention that the increased incidence of kidney tumours in males at the highest exposure concentration of 90 ppm (441 mg/m^3) in the 2-year inhalation bioassay in BDF1 mice (Yamamoto, 1996) was likely associated with regenerative cell proliferation.

Inhalation of chloroform for 4 days, 3 weeks, 6 weeks and 13 weeks produced an increase in labelling index in the epithelial cells of the proximal tubules of the renal cortex of F344 rats at doses above 30 ppm (147 mg/m^3) with daily exposure and at 90 ppm (441 mg/m^3) and above when exposure was for 5 days per week. Increases were similar in both male and female rats (Templin *et al.*, 1996b). Renal tumours have not been observed, however, in either sex of this strain exposed to up to 90 ppm (441 mg/m^3) in the only identified inhalation cancer bioassay (Yamamoto, 1996).

2.4.4 Humans

In general, chloroform elicits the same symptoms of toxicity in humans as in experimental animals. In humans, anesthesia may result in death due to respiratory and cardiac arrhythmias and failure. In humans, there have been infrequent reports of renal tubular necrosis and renal dysfunction resulting from the use of chloroform as an anesthetic (Kluwe, 1981). The lowest levels at which liver toxicity due to occupational exposure to chloroform has been reported are in the range of 80–160 mg/m^3 (with an exposure period of less than 4 months) in one study and in the range of 10–1000 mg/m^3 (with exposure periods of 1–4 years) in another study. The mean lethal oral dose for an adult is estimated to be about 45 g, but large interindividual differences in susceptibility occur (WHO, 1994).

Available epidemiological data do not allow conclusions to be drawn with respect to the potential carcinogenicity of chloroform in humans. There are some reports indicative of an association between exposure to DBPs in drinking water and increased risks of bladder cancer that fulfil, in part, traditional criteria of causality. However, there are some inconsistencies in the patterns of reported results between men and women and between smokers and non-smokers that are difficult to explain. Moreover, it is not possible to attribute these excesses specifically to chloroform (ILSI, 1997); indeed, due to the relative paucity of information on exposure in relevant studies, the sources of increased relative risks are unclear. Specific risks may be due to other DBPs, mixtures of by-products, other water contaminants or other factors for which chlorinated drinking-water or THMs may serve as a surrogate (WHO, in press).

3.0 PROPOSED ASSESSMENT OF "TOXIC" UNDER CEPA 1999

3.1 CEPA 1999 64(a): Environment

The environmental risk assessment of a PSL substance is based on the procedures outlined in Environment Canada (1997a). Environmental assessment endpoints (e.g., adverse reproductive effects on sensitive fish species in a community) are selected based on analysis of exposure pathways and subsequent identification of sensitive receptors. For each endpoint, a conservative Estimated Exposure Value (EEV) is selected and an Estimated No-Effects Value (ENEV) is determined by dividing a Critical Toxicity Value (CTV) by an application factor. A conservative (or hyperconservative) quotient (EEV/ENEV) is calculated for each of the assessment endpoints in order to determine whether there is potential ecological risk in Canada. If these quotients are less than one, it can be concluded that the substance poses no significant risk to the environment, and the risk assessment is completed. If, however, the quotient is greater than one for a particular assessment endpoint, then the risk assessment for that endpoint proceeds to an analysis based on more realistic assumptions, and the probability and magnitude of effects are considered. This latter approach involves a more thorough consideration of sources of variability and uncertainty in the risk analysis.

3.1.1 Assessment endpoints

In Canada, nearly all chloroform is released to air, but there are also some direct releases to surface water. Chloroform is also present in groundwater, particularly in the vicinity of landfills. Therefore, assessment endpoints for the environmental assessment of chloroform relate to populations of terrestrial animals living near industrial sources, freshwater pelagic organisms and groundwater-dwelling organisms.

3.1.2 Environmental risk characterization

3.1.2.1 Terrestrial organisms

Since chloroform does not bioaccumulate, biota are exposed via the atmosphere; given that the highest concentrations occur in air in cities, urban wildlife has the greatest potential for exposure to chloroform. Small mammals such as deer mice are likely to have the highest exposure because of their rapid respiration rate and high metabolism. Although no data have been identified for wild animals, data on effects are available for surrogates such as laboratory mammals.

For terrestrial wildlife exposed to chloroform via inhalation, the hyperconservative EEV is 110 $\mu g/m^3$, the highest atmospheric concentration of chloroform reported in the United States. This value is very conservative, because it is much higher than atmospheric concentrations reported for Canada. Chloroform in the atmosphere can be transported over long distances, but concentrations in Canada from this source would be much less than the EEV because of environmental transformation and dispersion.

The CTV is 9.8×10^3 μg/m^3, the lowest concentration of chloroform reported to cause adverse effects in inhalation toxicity tests with laboratory animals. Dividing this CTV by an application factor of 10 (to account for the extrapolation from laboratory to field conditions and interspecies and intraspecies variations in sensitivity) results in an ENEV of 9.8×10^2 μg/m^3.

The hyperconservative quotient is calculated as follows:

$$\text{Quotient} = \frac{\text{EEV}}{\text{ENEV}}$$

$$= \frac{110\ \mu g/m^3}{9.8 \times 10^2\ \mu g/m^3}$$

$$= 0.11$$

Because the hyperconservative quotient is less than one, it is unlikely that chloroform emissions will cause adverse effects on terrestrial wildlife in Canada.

A summary of the values used for the assessment of potential effects of chloroform on terrestrial wildlife is presented in Table 10.

3.1.2.2 Aquatic organisms

3.1.2.2.1 Freshwater pelagic biota

The highest levels observed in Canadian surface waters have in the past been near pulp and paper mills using chlorine bleaching. The maximum concentrations in the Fraser River below the Northwood Pulp and Timber outfall in 1989 and below the Canadian Pacific Forest Products Kraft Mill in Thunder Bay in 1986 were 83 μg/L and 200 μg/L, respectively. Chloroform concentrations in Canadian surface water samples collected after 1989 have been much lower. The maximum reported concentration of chloroform in 984 water samples collected from British Columbia, Alberta, Ontario and Quebec from 1990 to 1996 was 44 μg/L. This value will be used as the EEV.

Based on the available effects data, the most sensitive freshwater pelagic biota are early life stages of spring peepers. The 4-day post-hatching LC_{50} for the spring peeper was 0.27 mg/L, or 270 μg/L. Environment Canada (1997a) recommends estimating an EC_{25} or LC_{25} for the CTV and dividing by a factor of 10 to account for uncertainties arising from laboratory to field extrapolations and interspecies and intraspecies variations in sensitivity. Using an EC_{25} or LC_{25} ensures that the toxicity estimates are not model dependent, as is often the case with levels of effect below 5% (e.g., LC_1) (Moore and Caux, 1997). The 4-day post-hatch LC_{25} for spring peepers was 65.7 μg/L (95% CI = 36.6–106 μg/L). Dividing this value by 10 produces an ENEV of 6.57 μg/L.

The conservative quotient is calculated as follows:

$$\text{Quotient} = \frac{\text{EEV}}{\text{ENEV}}$$

$$= \frac{44\ \mu\text{g/L}}{6.57\ \mu\text{g/L}}$$

$$= 6.7$$

In order to determine the likelihood of chloroform causing harm to populations of freshwater pelagic organisms, it is necessary to examine the exposure and effects data more closely. From the 984 water samples collected from British Columbia, Alberta, Ontario and Quebec from 1990 to 1996, the 99th- and 95th-percentile chloroform concentration values were 2.94 µg/L and <1 µg/L, respectively. The median value was <0.2 µg/L. Only five of the samples contained chloroform concentrations above the ENEV value of 6.57 µg/L: three samples (44, 31.6 and 13 µg/L) were from Quebec, one sample (18 µg/L) was from British Columbia and one sample (7 µg/L) was from Alberta. Chloroform concentrations in Canadian surface water are therefore only rarely above the ENEV.

In the toxicity study with spring peepers, the LC_{50}, LC_{25}, LC_{10} and LC_1 were 270 µg/L, 65.7 µg/L, 17.7 µg/L and 1.9 µg/L, respectively. The LC_{10} can be used as a good representation of threshold mortality, given that acute toxicity test protocols allow 10% mortality in control treatments. Only 2 of the 984 water samples contained concentrations substantially above the LC_{10} value, and 1 sample contained chloroform at a concentration almost identical to the LC_{10} value. Other amphibians tested along with spring peepers were less sensitive. The LC_{10} for the second most sensitive amphibian (the leopard frog, *Rana pipiens*) was 383 µg/L. Other types of aquatic organisms (microorganisms, invertebrates and fish) were less sensitive still.

Based on the available information, concentrations of chloroform in Canadian surface waters are rarely above estimated toxicity thresholds for sensitive aquatic organisms. Chloroform therefore does not appear to pose significant risks to pelagic biota in Canada. A summary of the risk quotients for freshwater pelagic biota is presented in Table 11.

3.1.2.2.2 Groundwater-dwelling biota

No toxicity data were available for groundwater-dwelling biota. The only available toxicity data that could reasonably be extrapolated to effects on groundwater-dwelling biota are from studies on microbial populations used in wastewater treatment. Under anaerobic conditions, however, Yang and Speece (1986) observed inhibition of unacclimated cultures at 500 µg/L. Taking this value, 500 µg/L, as the CTV and dividing by an application factor of 10 produces a hyperconservative ENEV of 50 µg/L. This ENEV is uncertain, because no data were available to estimate effect levels for groundwater-dwelling invertebrates and because of the need to extrapolate from wastewater microbial populations to groundwater-dwelling populations. There are very few data available on the concentration of chloroform in groundwater not associated with the specialized conditions at a landfill site. In what may be regarded as typical of the groundwater conditions independent of the contamination found at landfill sites, 31 groundwater samples collected in British Columbia in 1987 and 1989 were all below the 1 μg/L detection limit (B.C. MOE, 1996). Furthermore, Carmichael (1996) reported a maximum concentration of 13.8 μg chloroform/L in 16 samples of B.C. groundwater collected in 1992 and 1993. Using 13.8 μg/L as the EEV, a conservative quotient would be calculated as follows:

$$\text{Quotient} = \frac{\text{EEV}}{\text{ENEV}}$$

$$= \frac{13.8\ \mu\text{g/L}}{50\ \mu\text{g/L}}$$

$$= 0.28$$

Therefore, it appears that chloroform poses little risk to groundwater-dwelling biota in Canada at locations that are not in the immediate vicinity of contaminated landfills.

Not surprisingly, the situation at some landfills in Canada is very different from the conditions existing in groundwater in general. These areas have been recognized as contaminated sites and are typically managed or have undergone remediation. They are atypical of the overall conditions that prevail and are therefore not suitable for use in assessing the impact of chloroform or other substances on the environment in general. For example, the maximum chloroform concentration first observed in groundwater at a landfill site in the Ottawa, Ontario, area in 1981 was 53 200 μg/L (Jackson *et al*., 1985). This site has since undergone extensive remediation, and, in 1988, the highest concentration of chloroform in groundwater from the same sampling site was 97.1 μg/L, while the concentration of chloroform at a sampling site approximately 50 m away was 5.8 μg/L (Moralejo, 1999). The highest concentrations reported at the other two contaminated sites mentioned in Section 2.3.2.5, 950 μg/L in leachates from a chemical company landfill near Sarnia, Ontario (King and Sherbin, 1986), and 916 μg/L in the groundwater at Ville Mercier, Quebec (Pakdel *et al*., 1992), were the primary figures used to determine the applicability for site remediation. Deriving quotients for these sites would not provide any further help in defining the risk that chloroform poses to the Canadian environment.

A summary of the values used for the assessment of potential effects of chloroform on groundwater-dwelling biota is presented in Table 10.

3.1.2.3 Discussion of uncertainty

There are a number of potential sources of uncertainty in this environmental risk assessment. Direct releases of chloroform from its use by industry are fairly well characterized. The quantity of chloroform released to the Canadian environment from wastewater treatment plants that chlorinate for disinfection is not known. Chloroform releases are highly variable, depending on the flow rate handled at the treatment plants and on the chemical conditions at the plants. Chloroform can be produced in the environment through reactions of chlorine with organic chemicals, and the quantity released from these sources is unknown.

High concentrations of chloroform were reported for surface waters in the vicinity of pulp and paper mills in the 1980s. Since that time, new government regulations have discouraged the use of elemental chlorine by these facilities, and it is believed that the release of chlorinated substances has dropped very significantly. For example, the total discharge of dioxins and furans from pulp and paper mills has fallen by approximately 99%. Concentrations of chloroform in water in the vicinity of pulp and paper mills have also likely decreased considerably, but there are few monitoring data available. According to Environment Canada's EEM database, chloroform was monitored in surface water in the vicinity of four mills in British Columbia. The concentration of chloroform was below the 1 μg/L detection limit in each of the 85 water samples (Environment Canada, 1999b).

Chloroform has been reported at quite high concentrations in leachate from landfills. These leachates could have the potential to contaminate groundwater and/or surface waters in the vicinity, but, again, data are lacking. Remediation work has been undertaken at some of these landfills, and the threat of pollution of groundwater and surface waters has been lowered considerably. Uncertainty also arises from the need to extrapolate effects data from wastewater microbial populations to groundwater-dwelling populations. However, it was shown that wastewater microbial organisms acclimated readily to chloroform and subsequently were able to tolerate concentrations of chloroform up to 15 mg/L.

Few studies have determined the toxicity of chloroform to terrestrial invertebrates, and the studies that do exist are not directly relevant for estimating potentially harmful concentrations in the soil. No information was found on the toxicity of chloroform to birds or wild mammals, but there are data on experimental animals.

3.2 CEPA 1999 64(b): Environment upon which life depends

The net chlorine loadings to the stratosphere from chloroform and its degradation products are small; therefore, chloroform is not considered to be an effective agent of stratospheric ozone depletion. The potential of chloroform to contribute to climate change and ground-level ozone formation is considered to be negligible. The magnitude of these effects would depend on the concentration of chloroform in the atmosphere, and, in Canada, concentrations of chloroform in air are low, usually less than 1 $\mu g/m^3$.

3.3 CEPA 1999 64(c): Human health

3.3.1 Estimated population exposure

As a basis principally to assess the relative contribution of various media and routes of exposure of the general population in Canada to chloroform, deterministic estimates of exposure were developed for six age groups. These were based on data on concentrations of chloroform in outdoor and indoor air acquired in national surveys in Canada and on estimates of the concentrations in foods in Canada and the United States, assuming age group-specific daily average rates of intake of these media (EHD, 1998). Although national surveys are available, estimates of intake in drinking water were based on monitoring data from the provinces and territories, which included much larger numbers of samples over an extended time frame. These data were also more representative of the water supplies of a larger proportion of the population and lead to more conservative estimates of intake, although they were collected and analysed by less consistent, less reliable and less comparable methodology than for the national surveys. Estimates of the average daily intake of chloroform by inhalation and dermal absorption during showering were also derived for teenagers, adults and seniors (Health Canada, 1999). Probabilistic estimates of exposure for various age groups of the general population were also developed based on distributions of the concentrations of chloroform in outdoor air, indoor air and drinking water in Canada from the same sources that served as the basis for the deterministic estimates. Age group-specific lognormal distributions of daily intake rates for these media were also assumed (EHD, 1998). Data were considered insufficient to develop probabilistic estimates of exposure from ingestion of foods or from showering (Health Canada, 1999).

3.3.1.1 Deterministic estimates of exposure to chloroform for the general population

Point estimates of the average daily intake (per kilogram body weight), based on these data (Section 2.3.2) and on reference values for body weight, inhalation volume and amounts of food and drinking water consumed daily, are presented for six age groups in Table 12. On this basis, average intake was estimated to range from 0.6 to 10.3 µg/kg-bw per day. The upper value in the range of estimated intakes (i.e., 10.3 µg/kg-bw per day) is for infants in the age group of 0–6 months and is based on the assumption that infants are exclusively formula fed during this period, with powdered infant formula reconstituted with tap water containing the maximum annual mean concentration of chloroform (i.e., 89.4 µg/L) as determined from provincial/territorial data. If it is assumed instead that infants are fed table-ready foods containing the same concentrations of chloroform as assumed for the remaining five age groups, the estimated average daily intakes for infants are much lower, ranging from 0.2 to 1.1 µg/kg-bw per day; for the six age groups, the average daily intakes then range from 0.2 to 6.9 µg/kg-bw per day, as indicated in Table 12.

Upper bounding estimates of the daily intake (per kilogram body weight), based on the maximum reported concentrations of chloroform in indoor and outdoor air and in drinking water in Canada and on the maximum reported concentrations in foods in Canada and/or the United States, have also been developed. These are also based on the reference values for body weight, inhalation volume and amounts of food and drinking water consumed daily (EHD, 1998) and are presented for six age groups in Table 13. On this basis, upper bounding estimates of daily intake range from 40 to 95 µg/kg-bw per day. It is assumed that infants are fed table-ready foods only and that their average intake of total tap water is 0.3 L/day (EHD, 1998). If it is assumed instead that infants are exclusively formula fed and that powdered infant formula is prepared with tap water containing the maximum reported concentration in Canada (i.e., 1224 µg/L), the upper bounding estimate of total daily intake for infants is more than twice as high (i.e., 147.6 µg/kg-bw per day, with 130.6 µg/kg-bw per day resulting from ingestion of total tap water).

The contribution of outdoor air to the estimates of average total daily intakes (i.e., Table 12) is considerably less than the contributions from indoor air, food and water, which are approximately similar in magnitude. The contributions of outdoor air and food to the upper bounding estimates of total daily intake (i.e., Table 13) are considerably less than the contributions from indoor air and tap water. On the basis of these deterministic estimates, the main pathways of exposure to chloroform for the general population in Canada are inhalation of indoor air and ingestion of tap water. It is also apparent from these deterministic estimates that the average daily intake from a single daily 10-minute shower can exceed the intake from all other exposure pathways.

3.3.1.2 Probabilistic estimates of exposure to chloroform for the general population

Probabilistic estimates of daily intake of chloroform by six age groups of the general population of Canada were generated in an Excel™ (Microsoft Corporation, 1997) spreadsheet using Crystal Ball™ (Decisioneering, Inc., 1996). Age group-specific body weights and rates of intake of air and tap water were assumed to be lognormally distributed and are characterized by their geometric means and standard deviations (EHD, 1998). A normal distribution of hours per day spent outdoors is assumed, characterized by an arithmetic mean and standard deviation of 3.0 ± 2.0 hours (EHD, 1998) and truncated at 0 and 9 hours. The same distribution is assumed for each of the age groups (Health Canada, 1999).

Two scenarios were developed for estimating daily intakes from exposure to chloroform in outdoor and indoor air and tap water. In a scenario for general population exposure, the following distributions of concentrations were assumed. For outdoor air, this was based on the distribution of chloroform in the air of 8807 samples collected during the 1990s in the NAPS program (Dann, 1998). For indoor air, it was based on the estimated geometric mean and standard deviation of an assumed lognormal distribution of chloroform in the indoor air of 754 Canadian homes (Concord Environmental Corporation, 1992; Health Canada, 1999). For tap water, the distribution of chloroform in the treated drinking water of 6607 samples, based on provincial/territorial data, was assumed.

In an RWC exposure scenario, the following distributions of concentrations were assumed. For outdoor air, the distribution of chloroform in air was that in 800 samples collected during the 1990s from four sites adjacent to major roadways in the NAPS program (Dann, 1998). For indoor air, this was again based on the estimated geometric mean and standard deviation of an assumed lognormal distribution of the concentrations of chloroform in the indoor air of 754

Canadian homes (Concord Environmental Corporation, 1992; Health Canada, 1999), since these data were inadequate as a basis to define a subset of concentrations for use in the RWC scenario. For tap water, the distribution of chloroform in the treated drinking water of 2597 samples, based on data from Manitoba and Alberta only where reported concentrations were highest, was assumed.

Simulations of 10 000 trials were run five times each using two sampling methods (i.e., Monte Carlo random and Latin Hypercube) to gauge the reproducibility of the parameters estimated. For the average population scenario, the 95th percentiles of the distribution of intakes from inhalation and ingestion of drinking water for five age groups of the general population (i.e., 0.5 years to 60+ years of age) range from 4.9 to 12.9 µg/kg-bw per day (Health Canada, 1999). Similar estimates were obtained from each of the two sampling methods. The relative standard deviations (for $n = 5$ simulations of 10 000 trials each) of the upper-percentile estimates of intake did not exceed 5%, indicating a high degree of reproducibility.

For the RWC scenario, the 95th percentiles of the distribution of intakes from inhalation and ingestion of drinking water for five age groups of the general population (i.e., 0.5 years to 60+ years of age) range from 7.0 to 19.1 µg/kg-bw per day (Health Canada, 1999). Similar estimates were obtained from each of the two sampling methods. The relative standard deviations (for $n = 5$ simulations of 10 000 trials each) of the upper-percentile estimates of intake did not exceed 7%, indicating a high degree of reproducibility.

For both the population exposure and RWC scenario, due to limitations of the data concerning the daily intake rate of total tap water by infants (EHD, 1998), probabilistic estimates could not be developed for the sixth age group (i.e., birth to 0.5 years).

3.3.2 Hazard characterization

As indicated in Section 2.4.4, available data on the toxicity of chloroform to humans contribute to our understanding of its toxicity to the extent that the target organ in populations exposed occupationally to high concentrations is similar to those in experimental animals (i.e., the liver).

The weight of evidence indicates that chloroform may be carcinogenic only at concentrations that induce the obligatory precursor lesions of sustained cytotoxicity and persistent proliferative regenerative response. For consistency with other assessments and for ease of presentation, cancer and non-cancer effects are considered separately here, although it is recognized that, based on consideration of mode of action, they are inextricably linked.

3.3.2.1 Cancer

As described in Section 2.4.3.7, there is considerable information available concerning the potential mode of induction of liver and kidney tumours by chloroform. This includes a range of metabolic studies. In addition, while there have been no cancer bioassays in which a range of intermediate endpoints has been investigated, proliferative response in target organs has been examined in numerous subsequent investigations following exposure via regimens similar to those in the long-term studies. The histopathology in the target organ for one of the more critical studies has also been reexamined (Hard *et al.*, in press). These data have been generated to investigate primarily the hypothesized mode of action for tumour induction in rodents that requisite precursor steps to cancer are 1) metabolism of chloroform by the target cell population, 2) induction of sustained cytotoxicity by metabolites and 3) subsequent persistent regenerative cell proliferation.

Metabolism to phosgene, resulting from the oxidative pathway that predominates at low exposures, is believed to be the principal determinant of sustained toxicity and resulting persistent proliferation that is hypothesized to lead to a higher probability of spontaneous cell mutation and subsequent cancer. Measures of cytotoxicity include histopathological effects and release of hepatic enzymes and labelling indices as surrogates for regenerative cell proliferation.

Chloroform causes liver and kidney tumours in mice and kidney tumours in rats. Although the hypothesized modes of induction of these tumours are similar, the weight of evidence varies considerably, and, as a result, they are addressed separately here.

Liver tumours are observed in B6C3F1 mice following administration of bolus doses by gavage in corn oil (NCI, 1976), but not following administration of the same daily doses in drinking water (Jorgenson *et al.*, 1985). That dose rate is a critical determinant of tissue damage (e.g., being greater following bolus dosing by gavage compared with continuous administration) is consistent with the proposed mode of induction of tumours. Doses at which tumours have been observed following administration in corn oil in the cancer bioassay are associated in shorter-term studies with sustained proliferative response in the liver of the same strain exposed similarly (Larson *et al.*, 1994c; Pereira, 1994; Melnick *et al.*, 1998). Sustained increases in proliferative response have not been observed following ingestion in drinking water of concentrations that did not induce increases in hepatic tumour incidence in the long-term bioassay (Larson *et al.*, 1994a).

The incidence and severity of hepatic necrosis in the mouse liver have been related to the degree of covalent binding of chloroform metabolites to tissue proteins. The linking of metabolism to toxicity is underscored by localization of covalent binding to the necrotic lesions and the predictable variations in toxic response produced by pretreatment with inducers or inhibitors of cytochrome P450-mediated metabolism, specifically CYP2E1. There is strong recent evidence that it is the oxidative metabolites specifically that predominate at low concentration and cause cytotoxicity in the mouse liver. This includes observation of a direct correlation between binding to the polar heads of phospholipid molecules (caused by oxidative metabolites) and protein binding in the liver of the strain of mice in which tumours have been observed (Ade *et al.*, 1994). Particularly strong evidence of the role of CYP2E1 in the induction of mouse liver tumours is also provided by recent studies in CYP2E1 null mice. There was no cytotoxicity or cell proliferation in the liver of two strains of CYP2E1 null mice (Sv/129 and B6C3F1 strains) at a concentration that caused severe hepatic lesions in the wild type of either strain (Constan *et al.*, 1999). There is a consistent association between CYP2E1 distribution, chloroform metabolism, pattern of covalent tissue binding and toxic injury to hepatocytes in mice.

Evidence of concordance between metabolism to reactive intermediates, cytotoxicity, regenerative proliferation and tumour development in the mouse liver is, therefore, very strong. Indeed, there is a wealth of information that indicates a relationship between sustained enhanced proliferative response and induction of liver neoplasia in the strain in which tumours have been observed (B6C3F1 mice).

Chloroform also induces renal tumours in BDF1 mice following inhalation (Yamamoto, 1996) and in ICI mice exposed by gavage in toothpaste (Roe *et al.*, 1979), although at lower rates than liver tumours. The response is strain and sex specific, occurring only in males.

Evidence of concordance between metabolism to reactive intermediates, cytotoxicity, regenerative proliferation and tumour development in the mouse kidney, although strong, is not as robust as for the mouse liver, due primarily to the more limited data available on sustained enhanced proliferative response in the strains in which tumours have been observed. Indeed, this is limited to a single study in BDF1 mice, in which there was an increase in labelling index in the kidneys of males but not females at concentrations that induced renal tumours in this strain in the long-term inhalation bioassay (Templin *et al.*, 1996c; Yamamoto, 1996). The available data concerning the relationship between sustained cellular proliferation and induction of renal tumours in another strain (B6C3F1) of mice indicate that sustained proliferative response is not always associated with tumours. In this strain, in shorter-term studies, there were sustained proliferative responses at doses at which kidney tumours were not observed in the relevant cancer bioassays following exposure by both gavage in corn oil and drinking water (NCI, 1976; Jorgenson *et al.*, 1985; Larson *et al.*, 1994a,c).

In mice, covalent binding of chloroform to renal proteins and microsomes is correlated with the degree of renal tubular necrosis, with strain and sex differences in sensitivity to nephrotoxicity being correlated with the ability of the kidney to metabolize chloroform. Similar to the liver, there is strong recent evidence that it is the oxidative metabolites specifically that predominate at low concentration and cause cytotoxicity in the mouse kidney. This includes observation of a direct correlation between binding to the polar heads of phospholipid molecules (caused by oxidative metabolites) and protein binding in the kidney of DBA/2J mice (Ade *et al.*, 1994). Particularly strong evidence of the role of CYP2E1 in the induction of mouse renal tumours is also provided by recent studies in CYP2E1 null mice. There was no cytotoxicity or cell proliferation in the kidney of two strains of CYP2E1 null mice (Sv/129 and B6C3F1 strains) at a concentration that caused severe hepatic lesions in the wild type of either strain (Constan *et al.*, 1999).

The weight of evidence for the hypothesized mode of induction of tumours in the rat kidney is considerably less than that for the mouse liver and kidney due primarily to limited data on intermediate endpoints in the only strain (Osborne-Mendel) in which increases in kidney tumours have been observed. These increases have been reported following exposure via both gavage in corn oil and drinking water (NCI, 1976; Jorgenson *et al.*, 1985). There are also few identified data on the relationship between the metabolism of chloroform and induction of renal lesions in rats. In the F344 rat, there were sustained increases in proliferative response in shorter-term studies following administration of doses similar to those that induced tumours in Osborne-Mendel rats following administration by gavage in corn oil but not following ingestion in drinking water (Larson *et al.*, 1995a,b). However, there are no bioassays in this strain following ingestion for direct comparison with these results. Sustained increases in labelling index were observed in the proximal tubules of F344 rats exposed to daily doses of 30 ppm (147 mg/m^3) and greater and at 90 ppm (441 mg/m^3) and greater at 5 days per week (Templin *et al.*, 1996b). However, increases in kidney tumour incidence were not observed in this strain exposed to up to 90 ppm (441 mg/m^3) for 5 days per week in the only inhalation cancer bioassay (Yamamoto, 1996).

Based on studies conducted primarily in F344 rats in which tumours have not been observed, a mode of action for carcinogenicity in the kidney observed in the carcinogenesis bioassay in Osborne-Mendel rats based on cytotoxicity and tubular cell regeneration is, therefore, plausible. For Osborne-Mendel rats, the results of reanalyses of the original renal tissues (Hard and Wolf, 1999; Hard *et al.*, in press), from both the drinking water bioassay (Jorgenson *et al.*, 1985) and the gavage study (NCI, 1976), have been critical. They provide strong support for the contention that the mode of induction of these tumours is consistent with the hypothesis that sustained proximal tubular cell damage is a requisite precursor lesion for chloroform-induced tumours.

In all cases where examined, therefore, sustained cytotoxicity and cellular proliferation were observed in the liver and kidney of the same strain of mice and rats exposed in a similar manner in short-term studies to concentrations or doses that induced tumours in these organs in cancer bioassays. However, the converse is not always true. Tumours have sometimes not been observed in cases where there have been sustained increases in damage and resulting proliferation in the same strain exposed to similar concentrations in the same manner in shorter-term studies, namely kidney lesions in B6C3F1 mice and F344 rats. These results are consistent with the hypothesis that, where chloroform causes tumours, toxicity and reparative hyperplasia are obligatory precursor steps. Tumours would not necessarily be expected whenever there is an increase in cell replication. The multiple susceptibility factors that produce tumours following cytotoxicity will depend on tissue-specific factors and will likely vary between species and strains. For example, in spite of the overt toxicity and sustained increased cell proliferation in the epithelial tissue of the nose in both rats and mice, no tumours have been noted in this tissue in any chronic studies, including the inhalation bioassay in which nasal tissues were carefully evaluated (Yamamoto, 1996).

The organs in which chloroform-induced cytotoxicity and proliferative lesions are observed (liver, kidney and nasal passages) correlate well with the distribution of CYP2E1 both across and within species (Löfberg and Tjälve, 1986). This consistent pattern of response to chloroform across species and organs supports a conclusion that chloroform-induced neoplasia is dependent on cytotoxicity coupled with regenerative cell proliferation. This is further supported by the considerable weight of evidence indicating that chloroform is not genotoxic, with unconvincing evidence for direct DNA reactivity. Due principally to limitations of the available data, though, weak genotoxicity in the rat cannot be precluded, which detracts somewhat from the weight of evidence in this species, although it is unknown whether this might be a result of secondary effects on DNA.

The hypothesized mode of carcinogenesis for chloroform is in keeping with the growing body of evidence supporting the biological plausibility that prolonged regenerative cell proliferation can be a causal mechanism in chemical carcinogenesis. This has been addressed in numerous articles, including Ames and Gold (1990, 1996), Cohen and Ellwein (1990, 1991, 1996), Preston-Martin *et al.* (1990), Ames *et al.* (1993), Tomatis (1993), Cohen (1995), Cunningham and Matthews (1995), Butterworth (1996), Farber (1996) and Stemmermann *et al.* (1996). Enhanced cell proliferation can lead to an increased frequency of spontaneous genetic damage either through errors that result from the infidelity of DNA replication or through the increased conversion of endogenous DNA changes into heritable genetic changes (Cohen and Ellwein, 1990, 1991, 1996; Ames *et al.*, 1993; Cohen, 1995). Additionally, during periods of cell replication, heritable non-mutagenic modifications of the genome may occur that may lead to changes in gene expression, contributing to carcinogenesis (U.S. EPA, 1996b). This view that cell proliferation is a risk factor for carcinogenesis is not universally accepted, because strict correspondence between increased cell turnover and carcinogenic response is not always demonstrable (Melnick, 1992; Farber, 1996). However, as indicated above, in view of the complex interplay of factors involved in the carcinogenesis process, it is not surprising that acute measures of cell proliferation do not always indicate a one-to-one correlation. Among the factors to be considered are the kinetics of DNA adduct formation and repair, the balance between cell proliferation, differentiation and death, proliferation in the target cell compartments compared with that of non-target cells and the consequences of overt tissue toxicity.

While the evidence is fairly convincing that chloroform is active principally through cytotoxic effects of phosgene and other products of oxidation, several other possibilities in which mutagenicity might play a role were also considered. One is that the effects of chloroform are a composite of those of metabolites from both oxidative and reductive pathways contributing to toxicity and carcinogenicity. However, several observations strongly support the predominant role of oxidative pathways in chloroform toxicity and make any significant role of reductive metabolism highly unlikely. Firstly, the macromolecular binding following administration of chloroform represents only a very small portion of the delivered dose. Secondly, the mechanisms of action related to the nature of the necrotic lesion, the time course of injury after single doses and the differences in cumulative damage on multiple exposures are very different for chloroform and carbon tetrachloride, the latter a compound for which the free radical (reductive) pathway is causative for toxicity. In addition, carbon tetrachloride, which is largely metabolized to a free radical, is not itself mutagenic. Based on these considerations, it was concluded that free radicals do not play a significant role in the toxicity or carcinogenicity of chloroform.

Another possibility is that minor pathways, associated with glutathione conjugation, produce mutagenic metabolites, as is believed to be the case for dichloromethane. However, there is little evidence for a significant direct conjugation pathway for chloroform. In studies with *Salmonella* tester strains with glutathione transferase T1-1 inserted into the bacterial genome and expressed during testing, a small increase in mutagenic activity (less than a factor of 2) was noted for chloroform at very high doses, even though positive controls with methylene chloride and bromochloromethane produced much larger responses (Pegram *et al.*, 1997). Neither of these other two potential modes of action is believed to play a significant role in the observed toxicity and carcinogenicity of chloroform, although further investigation of weak genotoxicity in the rat is desirable.

In summary, then, chloroform has induced liver tumours in mice and renal tumours in mice and rats. The weight of evidence of genotoxicity, sex and strain specificity and concordance of cytotoxicity, regenerative proliferation and tumours are consistent with the hypothesis that marked cytotoxicity concomitant with a period of sustained cell proliferation likely represent a secondary mechanism for the induction of tumours following exposure to chloroform. This is consistent with a non-linear dose–response relationship for induction of tumours. This cytotoxicity is primarily related to rates of oxidation of chloroform to reactive intermediates, principally phosgene and hydrochloric acid. The weight of evidence for this mode of action is strongest for hepatic and renal tumours in mice and more limited for renal tumours in rats.

3.3.2.2 Non-neoplastic effects

Effects observed most consistently at lowest concentrations or doses following repeated exposures to chloroform in rats and mice are cytotoxicity and regenerative proliferation. As discussed in relation to cancer, target organs are the liver (centrilobular region) and kidney (cortical region). In addition, chloroform has induced nasal lesions in rats and mice exposed by both inhalation and ingestion at lowest concentrations or doses.

Effects on the hematological, neurological and immunological systems have been reported less consistently and only at concentrations higher than those reported to induce effects on the liver, kidney and nose. Teratogenic effects have not been reported. Developmental/reproductive effects have been restricted to those observed most often at dose levels that caused other manifestations of systemic toxicity in the same studies, primarily hepatic effects. Such effects have also only been observed at doses greater than the lowest values reported in other studies to induce effects on the liver, kidney or nose.

3.3.3 Exposure–response analyses

While the target organ in populations exposed occupationally to high concentrations of chloroform is similar to those in experimental animals (i.e., the liver), the levels at which effects occur (i.e., dysfunction and necrosis) are not well documented and are inadequate as a basis to meaningfully characterize exposure–response.

3.3.3.1 Cancer

Available data are consistent with a mode of action for the carcinogenicity of chloroform that is a secondary consequence of cytotoxicity and associated reparative cell proliferation induced by oxidative metabolites. Hence, where chloroform causes tumours, oxidative metabolism, sustained cytotoxicity and persistent reparative hyperplasia are considered obligatory precursor steps. Based on this mode of action, the optimum approach to quantitation of exposure–response for comparison with estimated human exposure to draw conclusions concerning "toxic" as defined in Paragraph 64(c) of CEPA would be as follows. Data on non-cancer precursor events (cytotoxicity and regenerative proliferation) from interim kills in the critical cancer bioassay could be analysed on the basis of rates or amounts of oxidative metabolites produced per volume of tissue in the critical organ.

Liver tumours in mice (males, females) have been induced only by administration of bolus doses in corn oil (NCI, 1976) or at lethal concentrations (male and female mice) following inhalation (Yamamoto, 1996). Kidney tumours have been reported in male mice following ingestion in a toothpaste vehicle (Roe *et al*., 1979) or inhalation (Yamamoto, 1996), but at concentrations in the latter that cause severe kidney necrosis and acute lethality. Renal tumours in rats have been observed, however, in an adequate and relevant study in which the route and pattern of exposure were similar to those of humans (i.e., continuously in drinking water) (Jorgenson *et al*., 1985).

The critical carcinogenesis bioassay for quantitation of exposure–response for this assessment is, therefore, that of Jorgenson *et al*. (1985). Unfortunately, there were no data collected in this bioassay that might serve as the basis for quantitation of exposure–response for precursor lesions such as cytotoxicity or regenerative hyperplasia. A proportion of the slides from several dose groups was recently reexamined, however (Hard and Wolf, 1999; Hard *et al*., in press) (Section 2.4.3.4). While this reexamination confirmed histopathological changes consistent with the hypothesis that sustained tubular cytotoxicity and regenerative hyperplasia led to renal tubular tumour induction, data amenable for quantitation of exposure–response in this investigation were limited but permit at least crude comparison.[5]

There have been numerous subsequent shorter-term investigations of the proliferative response in the liver and kidney of various strains of mice and rats exposed to doses and concentrations of chloroform similar to those administered in the cancer bioassays in which tumours have been observed (Section 2.4.3.7.3). However, for renal tumours, most of these investigations have been conducted in the F344 rather than the Osborne-Mendel rat in which increases in renal tumours have been observed.

Limited available data indicate that the proliferative response in the F344 rat is not an appropriate surrogate for characterization of exposure–response for an intermediate endpoint for renal tumours in the Osborne-Mendel rat. For example, there is no indication of sex-specific variation in the proliferative response in the kidney of F344 rats (Larson *et al*., 1995a,b), although the increase in renal tumours in Osborne-Mendel rats is sex specific (i.e., restricted to males). In addition, in metabolic studies in F344 rats, intrarenal activation by cytochrome P450 was not implicated as a determinant of nephrotoxicity (Smith *et al.*, 1985). Available data are also inadequate as a basis of characterization of the relative sensitivity of the two strains to cytotoxicity. In the single study in which proliferative response was examined in Osborne-Mendel rats (Templin *et al.*, 1996a), it was concluded that they were about as susceptible as F344 rats to chloroform-induced renal injury, based on comparison 2 days following a single gavage administration. However, a statistically significant increase in labelling index was observed at a much lower dose (10 mg/kg-bw) in the Osborne-Mendel than in the F344 rat (90 mg/kg-bw). This latter observation may have been a function of the low value in controls for the Osborne-Mendel rats, attesting to the fact that these data are inadequate in themselves to characterize variations in sensitivity of the two strains. Rather, the results of this study contribute inasmuch as they are not inconsistent with a mode of action of induction of tumours involving tubular cell regeneration in Osborne-Mendel rats.

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[5] The incidence of histological changes indicative of tubular injury in slides from the animals sacrificed at 2 years was 0, 0, 50 and 100% for the untreated controls, 400 mg/L, 900 mg/L and 1800 mg/L dose groups, respectively.

Since quantitative data on the incidence of precursor lesions for cancer in the strain of interest are inadequate to meaningfully characterize exposure–response, a tumorigenic concentration has been developed for this purpose, based on the incidence of tubular cell adenomas and adenocarcinomas in the bioassay of Jorgenson *et al.* (1985) (Section 2.4.3.4).

In view of the weight of evidence for the role of oxidative metabolites in induction of requisite damage and resulting tumours, dose–response for cancer for chloroform is optimally expressed in terms of amounts or rates of formation of reactive metabolites in the target tissue. These rates have been estimated pharmacokinetically based on models that include specific parameters related to metabolic rates, enzyme affinities and enzyme tissue distribution (Section 2.4.3.7.2).

Characterization of exposure–response for cancer associated with exposure to chloroform in the context of rates of formation of reactive metabolites in the target tissue is considered appropriate in view of the sufficiency of the evidence to support the following assumptions inherent in the PBPK modelling:

1. In both experimental animals and humans, metabolism of chloroform by CYP2E1 is responsible for production of the critical reactive metabolite, phosgene.
2. The ability to generate phosgene and phosgene hydrolysis products determines which tissue regions in the liver and kidney are sensitive to the cytotoxicity of chloroform.
3. This dose–effect relationship is consistent within a tissue, across gender and across route of administration, and it may also be consistent across species.

Although several PBPK models in animals have been developed previously for chloroform, a human component was not available. Results presented here are from the "hybrid" animal model of the ILSI Expert Panel (ILSI, 1997), which was revised for this assessment and developed to permit its extension to humans (Section 2.4.3.7.2) (ICF Kaiser, 1999).

Various dose metrics have been considered in exposure–response analyses for chloroform. ILSI (1997) investigated four dose metrics in their "hybrid" animal model (Section 2.4.3.7.2) in relation to the labelling indices (assumed to be representative of response for cytotoxicity, the intermediate endpoint in induction of cancer) in the liver and kidney of exposed F344 rats. As would be expected based on the hypothesized mode of action, the fit for two of these — namely, the total amount of phosgene produced and the maximum concentration of chloroform reached in each experimental dosing interval with proliferative response — was poor. Of the other two, the mean and maximum rates of phosgene production during each experimental dosing interval, the fit with the labelling indices was best for maximum rate (VRAMCOR) (ILSI, 1997). For the current assessment, maximum rate of metabolism per unit kidney cortex volume (VRAMCOR) and mean rate of metabolism per unit kidney cortex volume during each dose interval (VMRATEK) were considered.

Although similar, the fit of the data on tumour incidence for VRAMCOR ($p = 0.97$) was slightly better than that for VMRATEK ($p = 0.84$). However, human equivalent concentrations for the former could be developed only for the lower 95% confidence limit of the Tumorigenic Concentration$_{01}$ (TC$_{01}$), since the maximum rate of human metabolism in the kidney is less than that in the rat. The maximum rate of metabolism that can be achieved in the human kidney, based on metabolic parameters included in the model (approximately 8.1 mg/L per hour), was between the animal dose metrics associated with the Benchmark Concentration$_{01}$ (BMC$_{01}$) and the lower 95% confidence limit of the BMC$_{05}$.

The results of the exposure–response assessment presented here are, therefore, those for the combined incidence of renal adenomas and adenocarcinomas in Jorgenson *et al.* (1985) versus VMRATEK,[6] fit to the following model (Howe, 1995):

$$P(d) = q_0 + (1 - q_0) \cdot \left[1 - e^{-q_1 d - \ldots - q_k d^k} \right]$$

where *d* is dose, *k* is the number of dose groups in the study, *P*(*d*) is the probability of the animal developing the effect at dose *d* and $q_i > 0$, $i = 1, \ldots, k$ and d_0 are parameters to be estimated. The model was fit to the incidence data using THRESH (Howe, 1995), and the Benchmark Dose$_{05}$s (BMD$_{05}$s) were calculated as the concentration *D* that satisfies:

$$\frac{P(D) - P(0)}{1 - P(0)} = 0.05$$

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[6] Based on a model incorporating updated physiological parameters for the rat of Brown *et al.* (1997) and data on drinking water consumption of Yuan (1993).

Results of the model fitting are presented in Figure 1. The relevant measure of exposure–response, i.e., the mean rate of metabolism (VMRATEK) in humans associated with a 5% increase in tumour risk (TC_{05}) estimated on the basis of the PBPK model, is 3.9 mg/L per hour (95% lower confidence limit, 2.5, chi-square = 0.04, degrees of freedom = 1, P-value = 0.84). This dose rate would result from continuous lifetime exposure to 3247 mg/L in water or 30 ppm (147 mg/m^3) chloroform in air. Respective lower 95% confidence limits for these values are 2363 mg/L and 15 ppm (74 mg/m^3).

Although data on dose–response were less robust than those for the cancer bioassay, for comparison, a benchmark dose was developed for histological lesions in the kidney in the reanalysis of a subset of the slides from the Jorgenson *et al.* (1985) bioassay. Results of the model fitting are presented in Figure 1. The mean rate of metabolism (VMRATEK) in humans associated with a 5% increase in histological lesions characteristic of cytotoxicity is 1.7 mg/L per hour (95% lower confidence limit, 1.4, chi-square = 3.9, degrees of freedom = 2, P-value = 0.14). This dose rate would result from continuous lifetime exposure to 1477 mg/L in water or 6.8 ppm (33.3 mg/m^3) in air. These values are approximately 2-fold less than those presented above, based on the more robust data on tumour incidence.

3.3.3.2 Non-neoplastic effects

The results of repeated-dose toxicity studies in which effects were observed at the lowest concentrations are summarized by manner of administration in Tables 14, 15 and 16 for bolus dosing by gavage, continuous administration in drinking water and inhalation, respectively. For ease of comparison, in addition to expression as concentrations in the administered medium (for continuous administration by drinking water and inhalation), effect levels have also been converted to mg/kg-bw, based on assumed volumes for inhalation and ingestion of drinking water and body weights (Health Canada, 1994), with the exception of those studies in which effects were observed at site of contact (i.e., nasal lesions following inhalation).

Following exposure by inhalation, effects at the site of contact are limiting, with proliferation in the nasal passages being reported at concentrations as low as 2 ppm (9.8 mg/m^3) in both rats and mice for 6 or 7 hours a day for periods ranging from 4 to 7 days (Larson *et al.*, 1996; Templin *et al.*, 1996b). At 5 ppm (25 mg/m^3), ossification of the nasal septum was observed in BDF1 mice exposed for 5 days per week for 2 years (Yamamoto, 1996). At 10 ppm (49 mg/m^3), cell proliferation and histopathological lesions were reported in the nasal passages of rats exposed for 6 hours per day for 1–3 days and mice exposed for 6 hours per day for 4–7 days (Mery *et al.*, 1994; Templin *et al.*, 1996b); ossification of the nasal turbinates was reported in rats exposed to this concentration for 5 days per week for 2 years (Yamamoto, 1996). In one study (Larson *et al.*, 1994c), moderate hepatic changes were observed in mice exposed to 10 ppm (49 mg/m^3) for 6 hours per day for 7 days. At concentrations of 25–30 ppm (123–147 mg/m^3), effects on the kidney and liver in rats and mice, including increases in organ weights, histopathological lesions and increases in proliferation, are observed following exposure for periods ranging from 4 days to 6 months (Table 16).

Following administration in drinking water, renal effects were reported at the lowest doses in rats and mice, with hepatic effects observed at higher doses. Regenerative proliferation was observed following 3 weeks' exposure to 17 and 40 mg/kg-bw per day in rats and mice, respectively (200 mg/L in drinking water) (Larson *et al*., 1994a, 1995b). Histological alterations in the liver of F344 rats were reported at 58 mg/kg-bw per day after 4 days' exposure (Larson *et al*., 1995b) (Table 15).

In protocols with bolus administration, the weight of the liver was affected in rats at the lowest dose following gavage in corn oil for 4 days (10 mg/kg-bw per day), while at higher doses (34 mg/kg-bw per day), there were histological changes in the liver (Larson *et al*., 1995a,b). At 15 mg/kg-bw per day, fatty cysts in the liver were observed in dogs exposed to chloroform in toothpaste base in gelatin capsules 6 days per week for 7.5 years (Heywood *et al*., 1979). At 34 mg/kg-bw per day, effects upon kidney and liver were reported in mice (Larson *et al*., 1994c); proliferation and lesions in the olfactory epithelium were observed at this dose in rats (Table 14).

In summary, then, short-term exposure by inhalation resulted in cellular proliferation in nasal passages in rats and mice at concentrations as low as 2 ppm (9.8 mg/m^3), with ossification being observed at slightly higher concentrations following long-term exposure. In short-term studies, moderate hepatic changes were observed in mice at 10 ppm (49 mg/m^3); following both short- and long-term exposure to 25–30 ppm (123–147 mg/m^3), there were multiple adverse effects in the kidney and liver in both rats and mice in several studies. Following ingestion in drinking water, regenerative proliferation following short-term exposure of mice to doses as low as 17 mg/kg-bw has been observed. Following bolus dosing, increases in proliferation in the liver of rats have been observed following short-term exposure of rats at 10 mg/kg-bw per day and fatty cysts in the liver of dogs at 15 mg/kg-bw per day.

For oral exposure, therefore, lowest reported effect levels in various species for different endpoints are similar and occur following bolus dosing. One of the lowest dose levels at which effects on liver and kidney have been observed is that in dogs reported by Heywood *et al*. (1979). As a result, a PBPK model in dogs was developed for this assessment, since characterization of exposure–response for ingestion on the basis of this study is likely to be protective, although it should be considered in the context of an example, in view of the fact that effects on the liver of rodents have also been observed in a similar dose range.

Two dose metrics were investigated in exposure–response: the mean rate of metabolism per unit centrilobular region of the liver (VMRATEL) and the average concentration of chloroform in the non-metabolizing centrilobular region of the liver (AVCL2). The two dose metrics were selected in order to evaluate the possibility of the fatty cyst formation in the dogs being the result of the solvent effects of chloroform or effects of a reactive metabolite.

The incidence of fatty cysts in this study (Table 4) versus VMRATEL and AVCL2 was fit to the model in the manner described for the assessment of exposure–response for cancer described above. Results of the model fitting are presented in Figure 2. The fit of the data on the incidence of fatty cysts was better for VMRATEL ($p = 1$) than for AVCL2 ($p = 0.45$). Hence, fit supported the assumption that a metabolite rather than chloroform itself was responsible for the observed effects. The mean rate of metabolism per unit centrilobular region of the liver (VMRATEL) in humans associated with a 5% increase in fatty cysts estimated on the basis of the PBPK model is 3.8 mg/L per hour (95% lower confidence limit = 1.3, chi-square = 0.00, degrees of freedom = 1, P-value = 1.00). This dose rate would result from continuous lifetime exposure to 37 mg/L in water or 2 ppm (9.8 mg/m^3) in air. Respective lower 95% confidence limits for these values were 12 mg/L and 0.7 ppm (3.4 mg/m^3).

3.3.4 Human health risk characterization

The exposure of Canadians was compared with the tissue dose measures described above through modelling of tissue doses resulting from a 24-hour exposure scenario. This scenario included inhalation, ingestion and dermal absorption from one 10-minute shower, a brief washing-up period before retiring, discrete periods of food and water consumption and inhalation of chloroform at various concentrations. The scenarios were based on midpoint and 95th percentiles of concentrations in outdoor air (background and commuting), indoor air, air in the shower compartment, air in the bathroom after showering, tap water and food (Table 17). The greatest single contributor to chloroform exposure within the 24-hour period results from inhalation during showering, which also includes dermal absorption. The human model was run with concentrations and durations in the multimedia scenario presented in Tables 18 and 19. This resulted in an estimated tissue dose that was 1794 (lower 95% confidence limit, 570) times less than that associated with the TC for cancer. For non-cancer, the comparable margin for the BMD$_{05}$ was 591 (lower 95% confidence limit, 165).

Since the tumorigenic and benchmark doses for cancer and non-cancer, respectively, are based on metabolized dose, they adjust for kinetic differences between animals and humans. An appropriate uncertainty factor for derivation of a Tolerable Intake for both cancer and non-cancer effects would therefore be in the range of 25, i.e., 10 (for intraspecies variation in toxicokinetics and toxicodynamics) × 2.5 (for interspecies variation in toxicodynamics) (Health Canada, 1994). Hence, the margins between estimated exposure and tumorigenic and benchmark doses for cancer and non-cancer, respectively, for chloroform are considerably greater than that considered as appropriate as a basis for development of Tolerable Intakes. As a result, exposure of the general population is considerably less than the level to which it is believed a person may be exposed daily over a lifetime without deleterious effect.

The lowest concentrations reported to induce cellular proliferation in the nasal cavities of rats and mice in short-term studies (i.e., 2 ppm [9.8 mg/m^3]) were compared directly with the midpoint and 95th-percentile estimates of concentrations of chloroform in indoor air in Canada. These values were the same as those selected to run the human models for the kidney and liver. The midpoint and 95th-percentile estimates are 4298 and 1225 times less than the lowest value reported to induce a proliferative response in rats and mice (midpoint for indoor air = 2.28 µg/m^3, 95th percentile = 8.0 µg/m^3). Comparisons with midpoint and 95th-percentile estimates of concentrations during showering were considered unwarranted, since such exposures are intermittent and last for very limited periods of time during the day. Based on considerations similar to those mentioned above for cancer and non-cancer effects associated with ingestion of chloroform, these margins are considerably greater than that considered appropriate as a basis for development of a Tolerable Concentration.

3.3.5 Uncertainties and degree of confidence in human health risk characterization

For the principal source of exposure of at least the older age groups of the general population to chloroform (i.e., showering), uncertainty is introduced by the assumption that concentrations in the water at the shower head are similar to those in the incoming cold tap water. Based on limited data, the average concentrations in the warm water may be twice as high as that in the incoming cold water during the summer months and up to 4 times as high as that in the colder incoming water during winter months (Benoit *et al.*, 1997). Additional uncertainty is introduced by the assumption that the concentrations measured in the water treatment plants and distribution systems are representative of the concentrations at the consumers' taps, to which the general population is exposed. Available data indicate that average concentrations may be 50% higher at the most remote locations than at the water treatment plants, depending on the specific treatment processes used and other factors.

For indoor air, confidence in characterization of concentrations is less that that for other media due primarily to the limited number of homes sampled and lack of sensitivity of analysis in the available survey (Concord Environmental Corporation, 1992). Concentrations measured were less than the limit of detection in approximately 90% of the samples from 754 homes, although the approach adopted for estimation of levels in these samples for characterization of exposure is not considered to be unrealistic or overly conservative.

There is a moderate degree of confidence in the quantitative estimates of the average intake of chloroform in drinking water for the general population. As indicated in relation to the estimates of exposure during showering, some uncertainty is introduced by the assumption that the concentrations measured in the water treatment plants and distribution systems are representative of the concentrations at the consumers' taps, to which the general population is exposed. This database included over 10 000 samples analysed between 1985 and 1997. Although analyses were performed by a number of different laboratories, sampling and analytical methods were similar. Although similar dechlorinating preservatives were utilized, the pH of the preserved samples was not adjusted concomitantly, and hence there may have been some alteration in concentrations of chloroform during storage (Lebel and Williams, 1995). Uncertainty in the quantitative estimates of daily intakes of chloroform is also introduced by assuming daily rates of intake of total tap water, which includes tap water used to prepare beverages. Concentrations of chloroform in hot beverages (e.g., tea and coffee) are unlikely to be as high as the concentrations in the cold tap water used for their preparation, as chloroform rapidly volatilizes from tap water during heating and boiling.

Although it contributes minimally to total exposure, there is a moderate degree of confidence in the characterization of the concentrations of chloroform in ambient air in Canada, due to the magnitude and sensitivity of the monitoring data. This was based on a large data set of 24-hour average concentrations, measured across the country, throughout the 1990s (Dann, 1998). Samples were collected by a standardized protocol, on a cyclical basis, at a fixed network of atmospheric monitoring sites, for analysis by a single, specialized laboratory. Confidence in the data is increased by the observation that chloroform was detected with similar frequencies and at similar ranges of concentrations in the ambient air from rural areas situated in widely separated geographical locations of Canada. The observation that the frequencies of detection and concentrations of chloroform were higher at suburban and urban locations than in these rural areas is also consistent with what might be expected on the basis of proximity to sources. Some uncertainty is introduced by the locations of the monitors, which are not strictly representative of personal exposure.

Uncertainty is introduced into the estimates of the average daily intake of chloroform from ingestion of foods by the assumption that the limited Canadian data available for a specific food item are representative of the concentrations generally encountered by the general population when ingesting that food item. Additional uncertainty is introduced by the assumption that concentrations of chloroform measured in specific food items in the United States are similar to the concentrations in those food items in Canada. Also, the concentration of chloroform was assumed to be zero in all food items for which data are not available. Nevertheless, there is a high degree of certainty that chloroform is not highly concentrated in foods in Canada, since chloroform is only moderately lipophilic and does not significantly biomagnify in food chains.

Confidence in the quantitative estimates of daily intakes of chloroform by ingestion for infants is low. As no data were available concerning the presence or concentrations of chloroform in human breast milk in Canada, estimates of intake for exclusively breast-fed infants could not be developed. Uncertainty is introduced by the assumption that infants are exclusively formula fed, since data concerning the presence or concentrations of chloroform in concentrated (i.e., powdered or liquid) infant formula were not available. As a result, the concentration of chloroform in the reconstituted infant formula was assumed to be identical to the concentrations in the domestic water supply. Similar uncertainty is introduced when it is assumed that infants are fed table-ready foods, due to the limitations identified previously regarding the concentrations of chloroform in the majority of food items consumed daily in Canada.

With respect to the toxicity of chloroform, the degree of confidence that critical effects in animal species are well characterized in the available database is high. Indeed, in numerous investigations in experimental animals by various routes of exposure, effects on the kidney, liver and nose have been consistently observed at lowest doses. The nature of the effects has been similar and generally consistent with a mode of action that involves cellular degeneration and death and regenerative proliferation induced by oxidative metabolites.

The degree of confidence in the database that supports an obligatory role of sustained cytotoxicity in the carcinogenicity of chloroform is also high, although there are some uncertainties. Indeed, there are few compounds for which the supporting database in this regard is as complete, consistent and cohesive as it is for chloroform. The weight of evidence in this regard is strongest for hepatic and renal tumours in mice. The evidence is more limited for renal tumours in rats, primarily due to the relative paucity of data, in strains where tumours have been observed, on metabolism and intermediate endpoints and the relationship between them. Uncertainty could be reduced, therefore, by acquisition of additional information on metabolism, cytotoxicity and proliferative response in the strain in which tumours were observed (i.e., Osborne-Mendel rats) following long-term exposure to chloroform. Additional data on metabolism and chronic (e.g., 2-year) cytotoxicity/proliferative response in the kidneys of F344 rats might also have contributed to greater confidence in the hypothesized mode of action.

While the overall weight of evidence for the genotoxicity of chloroform is negative, on the basis of available data, weak genotoxicity in the rat cannot be precluded. It would be desirable, therefore, to investigate further the possible nature of the interaction of chloroform with DNA in rats. Another area that could be clarified by further work is whether any of the metabolites of chloroform are DNA reactive.

For the PBPK model, among those parameters considered in the sensitivity analysis to have most impact on output, uncertainty was greatest for the metabolic parameters particularly in the kidney and for humans. Additional *in vitro* data on the metabolism of chloroform in the human kidney and liver would be useful not only to reduce uncertainty in these values, but, if performed on tissues from a number of individuals, potentially to address the issue of variability across the human population. In particular, it would be desirable to clarify whether the same pathways of metabolism contribute to the potential for cytotoxicity in rodents and humans, specifically with respect to CYP2E1 and other P450 isozymes. Determination of the kinetic constants for the CYP2E1 and CYP2B1 isoforms *in vivo* is also desirable and could be addressed through comparative kinetic analysis of gas uptake curves in phenobarbital-induced CYP2E1 knockout and normal mice. For the PBPK model for dogs, the blood/air partition coefficient in this species was considered to be similar to that for the rat, although for small molecular weight chlorocarbons, these are normally higher in smaller species, possibly due to variations in binding to hemoglobin. Similarly, for this model, local rates of metabolism in the dog were based on hepatic and renal distribution of CYP2E1 in rats.

Characterization of exposure–response for both non-cancer and cancer is based on increased incidence of the relevant endpoints (both fatty cysts in dogs and renal tumours in rats) for a small number of doses. However, the dose at which non-cancer effects were observed in this study is similar to the lowest reported effect levels for proliferative response in target organs of other species.

3.4 Proposed conclusions

CEPA 1999 64(a): Based on available data, it has been concluded that chloroform is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity.

CEPA 1999 64(b): Based on available data, it has been concluded that chloroform is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger to the environment on which life depends.

CEPA 1999 64(c): Based on available data, it has been concluded that chloroform is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Proposed overall conclusion: Based on the information available, it is proposed that chloroform not be considered "toxic" as defined in Section 64 of CEPA 1999.

3.5 Considerations for follow-up (further action)

Since it is proposed that chloform not be considered "toxic" as defined in Section 64 of CEPA 1999, investigation of options to reduce exposure under CEPA 1999 is not considered a priority at this time. However, this is based upon current use patterns; thus, future releases of this compound should continue to be monitored to ensure that exposure does not increase to any significant extent.

In view of the fact that showering is estimated to be the single greatest contributor to total daily intake of chloroform from drinking water, measures to reduce uptake from this source will be most effective in minimizing exposure of the general public.

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Table 1: Chemical and physical properties of chloroform

Chemical or physical property	Value	Reference
Molecular formula	$CHCl_3$	WHO (1994)
Molecular weight (g/mol)	119.38	
Physical state	Clear, colourless liquid at 20°C and normal pressure	
Melting point (°C) at 101.3 kPa	–63.2	Howe-Grant (1991)
Boiling point (°C) at 101.3 kPa	61.3	
Density (g/cm^3)		Howe-Grant (1991)
0°C/4°C	1.526	
25°C/4°C	1.481	
60.9°C/4°C	1.408	
Water solubility (mg/L) at 25°C	7230	Bannerjee *et al.* (1980)
	9300	Verschueren (1983)
Solubility in other solvents	Miscible with ethanol, diethyl ether, acetone, benzene, carbon disulphide, solvent naphtha	Crookes *et al.* (1994)
Vapour pressure (Pa)		Howe-Grant (1991)
0°C	8130	
10°C	13 400	
20°C	21 280	
30°C	32 800	
Henry's law constant (Pa · m^3/mol)		
20°C	303.97	Nicholson *et al.* (1984)
24.8°C	371.86	Gossett (1987)
Octanol/water partition coefficient (log K_{ow})	1.97	WHO (1994) Crookes *et al.* (1994)
Organic carbon/water partition coefficient (log K_{oc})	1.44–2.79	Grathwohl (1990)

Table 2: Concentrations of chloroform in drinking water in Canada during the 1990s

Province or territory	**Period**	**No. of samples**	**Frequency of detection (%)**	**Mean conc. (µg/L)**	**Maximum conc. (µg/L)**
Newfoundland	1995–1996	51	100	9.6	29.8
New Brunswick	1994–1996	410	100	9.4	77.4
Quebec	1991–1995	165	95	51.9	440
Ontario	1991–1997	3332	98	35.0	390
Manitoba	1990–1995	832	94	89.4	1125
Alberta	1990–1997	1765	92	60.6	1224
Northwest Territories	1990–1992	52	75	27.5	258
All provincial/territorial data for 1990s		6607	96	47.3	1224

Table 3: Distributions of concentrations of chloroform in drinking water from provincial/territorial data

Data set	**No. of samples**	**Median conc. (µg/L)**	**Mean conc. (µg/L)**	**Percentiles (µg/L) of distributions**			
				90th	**95th**	**97.5th**	**99th**
All data	6607	22.8	47.3	117	166	218	283
RWC data	2597	48.0	69.8	164	220	273	374

Table 4: Fatty cyst incidence in chronic dog study (Heywood *et al.*, 1979)

Group	**No. of dogs examined histologically**	**No. of dogs with nodules in liver**	**No. of dogs with fatty cysts**	
			Occasional or minimal	**Moderate or marked**
Males				
30 mg/kg-bw per day	7	0	1	6
15 mg/kg-bw per day	7	1	0	6
Vehicle control	15	0	7	1
Untreated	7	1	2	0
Alternative non-chloroform toothpaste	8	0	2	0
Females				
30 mg/kg-bw per day	8	4	0	7
15 mg/kg-bw per day	8	1	2	3
Vehicle control	12	3	3	0
Untreated	5	1	1	0
Alternative non-chloroform toothpaste	7	1	0	0

Table 5: Summary of liver tumour response to chloroform (modified from ILSI, 1997)

Exposure	**Dose levels in protocol (mg/kg-bw)**[1]	**Dose**[2] **(mg/kg-bw)**[1]	**Duration (weeks)**	**Strain**	**Sex**	**Response (%)**[3]	**Reference**
Mouse							
Corn oil	0 138 277	138	78	B6C3F1	Male	27 (POS)[4]	NCI (1976)
Corn oil	0 238 477	238	78	B6C3F1	Female	74 (POS)[4]	NCI (1976)
Water	0 34 65 130 263	263	104	B6C3F1	Female	(Neg.)[5]	Jorgenson *et al.* (1985)
Inhalation	0 5 ppm 30 ppm 90 ppm	90 ppm	104	BDF1	Male	7	Yamamoto (1996)
Inhalation	0 ppm 5 ppm 30 ppm 90 ppm	90 ppm	104	BDF1	Female	8	Yamamoto (1996)
Toothpaste	0 17 60	17	104	ICI	Male	26	Roe *et al.* (1979)
Toothpaste	0 17 60	60	104	ICI	Female	(Neg.)[5]	Roe *et al.* (1979)
Rat							
Corn oil	0 90 180	180	111	O-M[6]	Male	6	NCI (1976)
Corn oil	0 100 200	200	111	O-M[6]	Female	–4	NCI (1976)
Water	0 19 38 81 160	160	104	O-M[6]	Male	(Neg.)[5]	Jorgenson *et al.* (1985)
Inhalation	0 ppm 10 ppm 30 ppm 90 ppm	90 ppm	104	F344	Male	(Neg.)[5]	Yamamoto (1996)
Inhalation	0 ppm 10 ppm 30 ppm 90 ppm	90 ppm	104	F344	Female	(Neg.)[5]	Yamamoto (1996)

Exposure	**Dose levels in protocol (mg/kg-bw)**[1]	**Dose**[2] **(mg/kg-bw)**[1]	**Duration (weeks)**	**Strain**	**Sex**	**Response (%)**[3]	**Reference**
Water	One dose level only	>100	185	Wistar	Male	(Neg.)[5]	Tumasonis *et al.* (1987)
Water	One dose level only	>150	185	Wistar	Female	25 (POS)[4,7]	Tumasonis *et al.* (1987)
Toothpaste	0 15 75 165	165	80	S-D[8]	Male	0	Palmer *et al.* (1979)
Toothpaste	0 15 75 165	165	80	S-D[8]	Female	0	Palmer *et al.* (1979)

[1] Unless otherwise specified.

[2] Lowest dose giving a positive response or highest dose giving a negative response.

[3] Percent increase of tumour rate over controls; ( ) for decrease over controls.

[4] POS = statistically significant increase in liver neoplasms.

[5] Actual tumour data not given.

[6] Osborne-Mendel rats.

[7] Treated animals survived longer than controls (185 vs. 145 weeks).

[8] Sprague-Dawley rats.

Table 6: Summary of kidney tumour response to chloroform (modified from ILSI, 1997)

Exposure	**Dose levels in protocol (mg/kg-bw)**[1]	**Dose**[2] **(mg/kg-bw)**[1]	**Duration (weeks)**	**Strain**	**Sex**	**Response (%)**[3]	**Reference**
Mouse							
Corn oil	0 138 277	138	78	B6C3F1	Male	–2	NCI (1976)
Corn oil	0 238 477	238	78	B6C3F1	Female	0	NCI (1976)
Water	0 34 65 130 263	263	104	B6C3F1	Female	(Neg.)[4]	Jorgenson *et al*. (1985)
Inhalation	0 ppm 5 ppm 30 ppm 90 ppm	30 ppm	104	BDF1	Male	14 (POS)[5]	Yamamoto (1996)
Inhalation	0 ppm 5 ppm 30 ppm 90 ppm	90 ppm	104	BDF1	Female	(Neg.)[4]	Yamamoto (1996)
Toothpaste	0 17 60	60	104	ICI	Male	21 (POS)[5]	Roe *et al.* (1979)
Toothpaste	0 17 60	60	104	ICI	Female	(Neg.)[4]	Roe *et al.* (1979)
Toothpaste	One dose level only	60	104	C57BL	Male	(Neg.)[4]	Roe *et al.* (1979)
Toothpaste	One dose level only	60	104	CBA	Male	(Neg.)[4]	Roe *et al.* (1979)
Toothpaste	One dose level only	60	104	CF/1	Male	(Neg.)[4]	Roe *et al.* (1979)
Corn oil	0 90 180	180	111	O-M[5]	Male	24 (POS)[6]	NCI (1976)
Corn oil	0 100 200	200	111	O-M[5]	Female	4	NCI (1976)
Water	0 19 38 81 160	160	104	O-M[5]	Male	13 (POS)[6]	Jorgenson *et al*. (1985)

Exposure	Dose levels in protocol (mg/kg-bw)[1]	Dose[2] (mg/kg-bw)[1]	Duration (weeks)	Strain	Sex	Response (%)[3]	Reference
Inhalation	0 ppm 10 ppm 30 ppm 90 ppm	90 ppm	104	F344	Male	(Neg.)[4]	Yamamoto (1996)
Inhalation	0 ppm 10 ppm 30 ppm 90 ppm	90 ppm	104	F344	Female	(Neg.)[4]	Yamamoto (1996)
Water	One dose level only	>100	185[4,7]	Wistar	Male	7	Tumasonis *et al.* (1987)
Water	One dose level only	>150	185[4,7]	Wistar	Female	0	Tumasonis *et al.* (1987)
Toothpaste	0 15 75 165	165	80	S-D[8]	Male	0	Palmer *et al.* (1979)
Toothpaste	0 15 75 165	165	80	S-D[8]	Female	0	Palmer *et al.* (1979)

[1] Unless otherwise specified.

[2] Lowest dose giving a positive response or highest dose giving a negative response.

[3] Percent increase of tumour rate over controls; ( ) for decrease over controls.

[4] Actual tumour data not given.

[5] Osborne-Mendel rats.

[6] POS = statistically significant increase in renal neoplasms.

[7] Treated animals survived longer than controls (185 vs. 145 weeks).

[8] Sprague-Dawley rats.

Table 7: Pertinent histopathological findings in kidneys of male Osborne-Mendel rats in drinking water bioassay by Jorgenson *et al.* (1985) (from Hard *et al.*, in press)

Group	**No. of months on test**	**Total in group**	**No. of rats examined**[1]	**Effective number evaluated for cytotoxicity**[2]	**Percentage of effective number with lesions**[3] **of chloroform cytotoxicity**	**Mean grade of chronic progressive nephropathy**[4]	**Percent renal adenomas and carcinomas reported by Jorgenson *et al.* (1985)**
Untreated control	24	330	43	24	0	3.6	1.3
	18	20	19	19	0	2.7	
	12	20	20	20	0	1.8	
	6	20	20	20	0	0.9	
Water-matched control	24	50	0				2.0
	18	18	18	18	0	1.4	
	12	19	19	19	0	1.1	
	6	19	19	19	0	0.9	
1800 ppm	24	50	49	46	100	0.9	14.0
	18	20	18	17	100	0.9	
	12	19	18	18	100	0.6	
	6	20	20	20	95	0.6	
900 ppm	24	50	48	48	50[5]	1.7	6.3
	18	20	19	10	58[5]	1.6	
	12	20	20	20	33[5]	1.0	
	6	20	20	20	25[5]	0.8	
400 ppm	24	150	40	40	0	2.9	2.7
	18	20	20	19	0	2.3	
200 ppm	24	330	0				1.3
	18	20	16	16	0	2.3	

[1] Discrepancy in numbers attributed to missing slides.

[2] Excludes rats in which autolysis, end-stage chronic progressive nephropathy or other diffuse disease process prevented evaluation of chloroform-related cytotoxicity.

[3] Histological changes indicative of tubule injury, e.g., nuclear crowding, cytoplasmic vacuolation and faint basophilia in the mid to deep cortex.

[4] Spontaneous, age-related chronic progressive nephropathy; scores by D.C. Wolf, U.S. EPA.

[5] Chloroform-associated lesions were of a much lower grade than at 1800 ppm.

Table 8: Overview of genotoxicity tests

Endpoint	Core test	Alternative test	Indicator test	Other
In vitro: gene mutation	negative 2 strains *Salmonella* (Uehleke *et al*., 1976, 1977)	negative V79 (Sturrock, 1977)		negative 1 strain yeast (Zimmermann and Scheel, 1981)
	negative 5 strains *Salmonella* (Simmon *et al*., 1977)	negative V79 (Kuroda, 1986)		negative 1 strain yeast (Sharp and Parry, 1981a)
	negative 5 strains *Salmonella* (Gocke *et al*., 1981)	negative V79 HGPRT (Hoechst, 1987)		negative 1 strain yeast (Jagannath *et al*., 1981)
	negative 5 strains *Salmonella* (Trueman, 1981)	equivocal V79 (Kuroda, 1986)		negative (reversion) 1 strain yeast (Mehta and von Borstel, 1981)
	negative 2 strains *Salmonella* (Ichinotsubo *et al*., 1981b)	weakly positive mouse lymphoma (Myhr and Caspary, 1988)		negative 1 strain yeast (Loprieno, 1981)
	negative 2 strains *Salmonella* (Venitt and Crofton-Sleigh, 1981)	positive mouse lymphoma (Mitchell *et al*., 1988)		negative 1 strain *Aspergillus* (Gualandi, 1984)
	negative 3 strains *Salmonella* (Gatehouse, 1981)			negative *Drosophila* (Gocke *et al*., 1981)
	negative 5 strains *Salmonella* (Baker and Bonin, 1981)			negative *Drosophila* (Vogel *et al*., 1981)
	negative 3 strains *Salmonella* (MacDonald, 1981)			equivocal 1 strain yeast (Sharp and Parry, 1981a)
	negative 3 strains *Salmonella* (Nagao and Takahashi, 1981)			equivocal 1 strain yeast (Loprieno, 1981)
	negative 5 strains *Salmonella* (Rowland and Severn, 1981)			positive 1 strain yeast (Callen *et al*., 1980)
	negative 3 strains *Salmonella* (Richold and Jones, 1981)			
	negative 5 strains *Salmonella* (Simmon and Shepherd, 1981)			
	negative 5 strains *Salmonella* (Brooks and Dean, 1981)			
	negative 5 strains *Salmonella* (van Abbé *et al*., 1982)			
	negative 2 strains *Salmonella* (vapour assay) (van Abbé *et al*., 1982)			
	negative 1 strain *Salmonella* (Skopek *et al*., 1981)			
	negative 1 strain *Salmonella* (LeCurieux *et al*., 1995)			
	negative 1 strain *E. coli* (Gatehouse, 1981)			
	negative 1 strain *E. coli* (Matsushima *et al*., 1981)			
	negative 2 strains *E. coli* (Kirkland *et al*., 1981)			

Endpoint	Core test	Alternative test	Indicator test	Other
	negative 2 strains *E. coli* (Venitt and Crofton-Sleigh, 1981)			
	negative 1 strain *E. coli* (Greim *et al*., 1977)			
	equivocal 1 strain *Photobacterium* (Wecher and Scher, 1982)			
	equivocal/negative 2 strains *Salmonella* (Hubbard *et al*., 1981)			
	equivocal/negative 4 strains *Salmonella* (Garner *et al*., 1981)			
	positive/weakly positive 4 strains *Salmonella* (Varma *et al*., 1988)			
	weakly positive 1 strain *Salmonella* (Pegram *et al*., 1997)			negative (aneuploidy) 1 strain yeast (Parry and Sharp, 1981)
				negative (mitotic crossing over) 2 strains yeast (Kassinova *et al*., 1981)
In vitro: cytogenetic			negative SCE Chinese hamster ovary (CHO) (White *et al*., 1979)	negative newt (LeCurieux *et al*., 1995)
			negative SCE CHO (Perry and Thomson, 1981)	positive Allium (Cortés *et al*., 1985)
			negative cell transformation hamster kidney (Daniel and Dehnel, 1981)	positive (deletions) 1 strain yeast (Brennan and Schiestl, 1998)
			negative cell transformation hamster kidney (Styles, 1979, 1981)	equivocal (aneuploidy) 1 strain yeast (Parry and Sharp, 1981)
			negative UDS rat hepatocyte (Althaus *et al*., 1982)	
			negative UDS mouse hepatocyte (Larson *et al*., 1994d)	
			negative UDS repair human hepatocyte (Butterworth *et al*., 1989)	

Endpoint	Core test	Alternative test	Indicator test	Other
			negative SCE human lymphocyte (Kirkland *et al.*, 1981)	
			negative UDS human lymphocyte (Perocco *et al.*, 1983)	
			negative UDS human lymphocyte (Perocco and Prodi, 1981)	
			negative UDS human HeLa (Martin and McDermid, 1981)	
			weakly positive SCE rat erythroblast (Fujie *et al.*, 1993)	
			positive SCE human lymphocyte (Morimoto and Koizumi, 1983)	
			positive SCE CHO (Athanasiou and Kyrtopoulos, 1981)	
			positive adenovirus transformation hamster embryo (Hatch *et al.*, 1983)	negative DNA damage 5 strains *E. coli* (Tweats, 1981)
			weakly positive cell transformation hamster kidney (Daniel and Dehnel, 1981)	negative DNA damage 5 strains *E. coli* (Green, 1981)
In vitro: genotoxicity			negative 1 strain *E. coli* (LeCurieux *et al.*, 1995)	negative DNA damage 2 strains *E. coli* (Rosenkranz *et al.*, 1981)
			negative chromosome breakage human lymphocytes (Kirkland *et al.*, 1981)	negative DNA damage 6 strains *E. coli* (Ichinotsubo *et al.*, 1981a)
			negative DNA and RNA adduct mouse liver (Diaz-Gomez and Castro, 1980)	negative induction of prophage in *E. coli* (Thomson, 1981)
			equivocal double strand breaks mouse hepatocytes (Ammann and Kedderis, 1997)	negative DNA damage in *Bacillus* (Kada, 1981)

Endpoint	Core test	Alternative test	Indicator test	Other
			equivocal strand breaks mouse hepatocytes (Enright, 1995)	negative DNA repair 2 strains yeast (Kassinova *et al.*, 1981)
			equivocal double strand breaks rat hepatocytes (Ammann and Kedderis, 1997)	weakly positive DNA damage 2 strains *E. coli* (Rosenkranz *et al.*, 1981)
			positive binding calf thymus DNA (DiRenzo *et al.*, 1982)	equivocal DNA repair 5 strains yeast (Sharp and Parry, 1981b)
			weak positive binding calf thymus DNA (Colacci *et al.*, 1991)	equivocal SCE Allium (Cortés *et al.*, 1985)
				positive DNA damage 6 strains *E. coli* (Ichinotsubo *et al.*, 1981a)
In vivo: gene mutation	negative micronuclei mouse polychromatic erythrocytes (PCE) in bone marrow (Tsuchimoto and Matter, 1981)	negative lacI transgenic mouse (Butterworth *et al.*, 1998)		
In vivo: cytogenetic	negative micronuclei mouse PCE (Gocke *et al.*, 1981)	negative sperm abnormalities mouse (Topham, 1980, 1981)		
	negative micronuclei mouse PCE (Salamone *et al.*, 1981)	positive sperm abnormalities mouse (Land *et al.*, 1981)		
	equivocal micronuclei mouse PCE (Agustin and Lim-Sylianco, 1978)	positive SCE mouse bone marrow (Morimoto and Koizumi, 1983)		
	positive micronuclei rat kidney (Robbiano *et al.*, 1998)			
	positive micronuclei rat liver (Sasaki *et al.*, 1998)			
	positive chromosomal aberration rat bone marrow (Fujie *et al.*, 1990)			
	weak positive chromosomal aberration hamster bone marrow (Hoechst, 1987)			
	negative UDS rat hepatocytes (Mirsalis *et al.*, 1982)			

Endpoint	Core test	Alternative test	Indicator test	Other
In vivo: genotoxicity	negative UDS mouse hepatocytes (Larson *et al.*, 1994d)		negative DNA methylation mouse liver (Pereira *et al.*, 1998)	
	equivocal aneuploidy mouse bone marrow (Sharma and Anand, 1984)		negative DNA strand breaks rat kidney (Potter *et al.*, 1996)	
			negative DNA binding mouse liver (Pereira *et al.*, 1982)	
			negative DNA adducts mouse liver (Diaz-Gomez and Castro, 1980)	
			negative DNA repair mouse liver (Reitz *et al.*, 1982)	
			negative DNA damage rat liver and kidney (Petzold and Swenberg, 1978)	
			equivocal "bimodal" DNA methylation mouse liver (Pereira *et al.*, 1998)	
			weak positive DNA binding rat liver and kidney (Pereira *et al.*, 1982)	
			weak positive DNA binding rat and mouse liver and kidney (Colacci *et al.*, 1991)	

Table 9: Physiological and metabolic parameter values used to exercise the physiologically based model

	Rat[1]	**Rat[2]**	**Dog**	**Human**
Weights (kg)				
Body	0.40	0.40	15.0	70.0
% of body weight				
Fat	0.063	0.124	0.145	0.2142
Kidney	0.0071	0.0073	0.0055	0.0044
Liver	0.0253	0.0366	0.0329	0.0257
Rapidly perfused	0.0439	0.0621	0.0836	0.0709
Slowly perfused	0.77	0.594	0.548	0.4368
Fractional tissue subvolumes (kg)				
Liver periportal (fraction of liver volume)	0.58	0.58	0.58	0.58
Liver centrilobular (fraction of liver volume)	0.42	0.42	0.42	0.42
Kidney cortical (fraction of kidney volume)	0.76	0.76	0.73	0.70
Kidney non-cortical (fraction of kidney volume)	0.24	0.24	0.27	0.30
Flows (L/h)				
Alveolar ventilation (L/h for 1-kg animal)	15.0	24.2	28.5	24.0
Cardiac output (L/h for 1-kg animal)	15.0	14.4	30.9	16.5
% of cardiac output				
Fat	0.05	0.07	0.07	0.052
Kidney	0.25	0.141	0.173	0.175
Liver	0.25	0.183	0.297	0.227
Slowly perfused	0.19	0.336	0.277	0.249
Partition coefficients				
Blood/air	20.8	20.8	20.8	7.43
Fat/air	203.0	203.0	203.0	280.0
Kidney/air	11.0	11.0	11.0	11.0
Liver/air	21.1	21.1	21.0	17.0
Rapidly perfused/air	21.1	21.1	21.0	17.0
Slowly perfused/air	13.9	13.9	13.9	12.0
Metabolic constants				
VmaxC for liver (mg/h for 1-kg animal)	6.44	6.44	11.025	15.7
Km for liver (mg/L)	0.543	0.543	0.4955	0.448
VmaxC for kidney (mg/h for 1-kg animal)	0.355 (0.094)[3]	0.067	0.078	0.089
Km for kidney (mg/L)	0.543	0.543	0.4955	0.448
Absorption rate constants for water (/h)				
kSL (from stomach)	2.5	2.5	NA	5.0
kIL (from upper gastrointestinal [GI] tract)	0.5	0.5	NA	0.0
kSI (rate constant from stomach to upper GI tract)	3.5	3.5	NA	0.0
Absorption rate constants for oil gavage (/h)				
kSL	1.5	1.5	1.5	NA
kIL	0.5	0.5	0.5	NA
kSI	1.8	1.8	1.8	NA

[1] Based on ILSI (1997).

[2] Updated with more recent information provided by Brown *et al.* (1997).

[3] Estimated using the equation provided by Corley *et al.* (1990).

Table 10: Summary of risk quotients for chloroform for CEPA 1999, 64(a)

Environmental compartment	**Estimated Exposure Value** **EEV**	**Critical Toxicity Value** **CTV**	**Application factor** **AF**	**Estimated No-Effects Value** **ENEV**	**Risk quotient** **EEV/ENEV**
Terrestrial wildlife	110 µg/m^3	9.8×10^3 µg/m^3	10	9.8×10^2 µg/m^3	0.11
Freshwater pelagic biota	44 µg/L	65.7 µg/L	10	6.57 µg/L	6.7
Groundwater biota	13.8 µg/L	500 µg/L	10	50 µg/L	0.28

Table 11: Summary of risk quotients for freshwater pelagic biota

EEV (µg/L)	Descriptor	CTV (µg/L)	Application factor	ENEV (µg/L)	Quotient (EEV/ENEV)
44	Maximum reported conc., 1990–1996	65.7 (4-day post-hatch LC_{25} — spring peepers)	10	6.57	6.7
2.94	99th percentile — all data, 1990–1996	65.7 (4-day post-hatch LC_{25} — spring peepers)	10	6.57	0.45
<1 µg/L	95th percentile — all data, 1990–1996	65.7 (4-day post-hatch LC_{25} — spring peepers)	10	6.57	<0.15
<0.2	Median — all data, 1990–1996	65.7 (4-day post-hatch LC_{25} — spring peepers)	10	6.57	<0.03
44	Maximum reported conc., 1990–1996	17.7 (4-day post-hatch LC_{10} — spring peepers)	1	17.7	2.5
2.94	99th percentile — all data, 1990–1996	17.7 (4-day post-hatch LC_{10} — spring peepers)	1	17.7	0.17
<1 µg/L	95th percentile — all data, 1990–1996	17.7 (4-day post-hatch LC_{10} — spring peepers)	1	17.7	<0.06
<0.2	Median — all data, 1990–1996	17.7 (4-day post-hatch LC_{10} — spring peepers)	1	17.7	<0.01

Table 12: Deterministic estimates of average daily intake of chloroform by the general population

Medium of exposure	**Average intake (µg/kg-bw per day) by various age groups in the general population**					
	0–6 months[1]	**7 months–4 years**[2]	**5–11 years**[3]	**12–19 years**[4]	**20–59 years**[5]	**60+ years**[6]
Outdoor air[7,8]	0.002–0.034	0.004–0.072	0.003–0.056	0.002–0.032	0.001–0.027	0.001–0.024
Indoor air[9]	0.559–0.744	1.197–1.596	0.933–1.244	0.531–0.708	0.456–0.608	0.396–0.528
Food[10]	–[11]	0.150–1.145	0.105–0.899	0.060–0.612	0.043–0.478	0.028–0.349
Water[12]	1.003–9.536	0.424–4.037	0.334–3.172	0.190–1.806	0.199–1.891	0.209–1.987
SUBTOTAL	1.56–10.31	1.78–6.85	1.38–5.37	0.78–3.16	0.70–3.00	0.63–2.89
Inhalation and dermal intake from daily showering[13]				0.43–4.06	0.36–3.40	0.35–3.35

[1] Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day and to consume 0.8 L of total tap water per day (EHD, 1998).

[2] Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day and to consume 0.7 L of total tap water per day (EHD, 1998).

[3] Assumed to weigh 31.0 kg, to breathe 14.5 m^3 of air per day and to consume 1.1 L of total tap water per day (EHD, 1998).

[4] Assumed to weigh 59.4 kg, to breathe 15.8 m^3 of air per day and to consume 1.2 L of total tap water per day (EHD, 1998).

[5] Assumed to weigh 70.9 kg, to breathe 16.2 m^3 of air per day and to consume 1.5 L of total tap water per day (EHD, 1998).

[6] Assumed to weigh 72.0 kg, to breathe 14.3 m^3 of air per day and to consume 1.6 L of total tap water per day (EHD, 1998).

[7] Based on the assumption that all age groups spend 3 hours outdoors per 24-hour day (EHD, 1998).

[8] Based on the range of annual site-specific (censored) mean concentrations in the NAPS data set (Dann, 1998), from 0.05 µg/m^3 (at NAPS site numbers 90601 [in 1994], 62601, 61901 and 90701 [in 1995] and 54401 [in 1996]) to 0.96 µg/m^3 (at NAPS site number 100127 [in 1992]). Data were censored by assuming a concentration equivalent to one-half the limit of detection (i.e., ½ × 0.1 µg/m^3 = 0.05 µg/m^3) for the concentration of chloroform in samples in which it was not detected (Health Canada, 1999).

[9] Based on censored mean concentrations of chloroform in indoor air from 754 Canadian homes in nine provinces (Concord Environmental Corporation, 1992). As chloroform was detected (at a concentration greater than the 3.5 µg/m^3 limit of detection) in only 10.7% of the samples collected, estimates of the arithmetic mean concentration were derived using different statistical approaches. A lower mean concentration (2.28 µg/m^3) was calculated on the assumption that concentrations in this data set are lognormally distributed (Walker, 1998). A higher mean concentration (3.04 µg/m^3) was calculated when a value equivalent to one-half the limit of detection (i.e., ½ × 3.5 µg/m^3 = 1.75 µg/m^3) was assumed for the concentration of chloroform in samples in which it was not detected (Health Canada, 1999).

[10] Estimates of intakes of chloroform from ingestion of foods are based on per capita arithmetic mean daily consumption rates (grams/day) from EHD (1998). Lower intake estimates are based on midpoint estimates of concentrations of chloroform in 16 food items measured in Canada. Higher intake rates are based on midpoint estimates of concentrations of chloroform in 131 food items measured in Canada and/or the United States. In the remaining food items, the concentration of chloroform is assumed to be zero.

[11] Infants are assumed to be exclusively formula fed. It is assumed that powdered infant formula is prepared using tap water containing concentrations of chloroform ranging from 9.4 to 89.4 µg/L (i.e., as in footnote 12), resulting in daily intakes of chloroform ranging from 1.00 to 9.54 µg/kg-bw per day. If it is assumed instead that infants eat the foods containing the concentrations of chloroform indicated in footnote 10, at the average daily rates of consumption indicated in EHD (1998), the estimated intakes are much lower, ranging from 0.21 to 1.13 µg/kg-bw per day.

[12] Estimates of intakes of chloroform from ingestion of drinking water are based on average daily consumption rates of "total tap water" from EHD (1998) and on the range of arithmetic mean concentrations of chloroform in drinking water, from 9.4 µg/L (in New Brunswick, for the period 1994–1996) to 89.4 µg/L (in Manitoba, for the period 1990–1995). Total tap water includes water used in the preparation of beverages. Average daily consumption rates of "tap water as drinking water" are also available in EHD (1998), and their use results in lower estimates of intakes of chloroform from ingestion of drinking water.

[13] Based on the assumption from Benoit *et al.* (1998) that the combined intake of chloroform from the inhalation and dermal routes during a 10-minute shower is equivalent on an annual average to the intake from ingestion of 2.7 L of cold tap water from the same source. It is assumed that this tap water contains concentrations of chloroform ranging from 9.4 to 89.4 µg/L (i.e., as in footnote 12).

Table 13: Upper bounding estimates of daily intake of chloroform by the general population

Medium of exposure	**Upper bounding estimates of intake (µg/kg-bw per day) by various age groups in the general population**					
	0–6 months[1]	**7 months–4 years[2]**	**5–11 years[3]**	**12–19 years[4]**	**20–59 years[5]**	**60+ years[6]**
Outdoor air[7,8]	0.21	0.45	0.35	0.20	0.17	0.15
Indoor air[9]	16.81	36.02	28.08	15.97	13.72	11.92
Food[10]	–[11]	2.87	2.36	1.58	1.25	0.89
Water[12]	130.6[11]	55.28	43.43	24.73	25.90	27.20
SUBTOTAL	147.6	94.62	74.22	42.48	41.04	40.16
Inhalation and dermal intake from daily showering[13]				55.64	46.61	45.90

[1] Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day and to consume 0.3 L of total tap water per day when eating table-ready foods (EHD, 1998).

[2] Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day and to consume 0.7 L of total tap water per day (EHD, 1998).

[3] Assumed to weigh 31.0 kg, to breathe 14.5 m^3 of air per day and to consume 1.1 L of total tap water per day (EHD, 1998).

[4] Assumed to weigh 59.4 kg, to breathe 15.8 m^3 of air per day and to consume 1.2 L of total tap water per day (EHD, 1998).

[5] Assumed to weigh 70.9 kg, to breathe 16.2 m^3 of air per day and to consume 1.5 L of total tap water per day (EHD, 1998).

[6] Assumed to weigh 72.0 kg, to breathe 14.3 m^3 of air per day and to consume 1.6 L of total tap water per day (EHD, 1998).

[7] Based on the assumption that all age groups spend 3 hours outdoors per 24-hour day (EHD, 1998).

[8] Based on the maximum 24-hour average concentration measured in the NAPS data set (i.e., 5.99 µg/m^3 at NAPS site 100127 in 1992). These data are from Dann (1998).

[9] Based on the maximum 24-hour average concentration of chloroform in indoor air (i.e., 68.6 µg/m^3) from among 754 Canadian homes in nine provinces (Concord Environmental Corporation, 1992).

[10] Estimates of intakes of chloroform from ingestion of foods are based on per capita arithmetic mean daily consumption rates (grams/day) from EHD (1998) and the maximum concentrations of chloroform in 131 food items measured in Canada and/or the United States. In the remaining food items, the concentration of chloroform is assumed to be zero. Infants are assumed to eat table-ready foods only, containing the maximum concentration of chloroform, as for the remaining six age groups.

[11] It is assumed that infants are exclusively formula fed; the estimate of the total daily intake by infants from ingestion of food and water is 130.6 µg/kg-bw per day. If infants are assumed to be fed table-ready foods, the estimate of the average daily intake of total.tap water is 0.3 L/day. Based on this assumption, the total daily intake by infants from ingestion of food and water is 51.2 µg/kg-bw per day. If infants are assumed to be exclusively breastfed a maximum of 1.033 L/day (EHD, 1998) of breast milk containing 65 µg chloroform/L (Erickson *et al.*, 1980), the estimate of total daily intake by infants from ingestion of food and water is 8.95 µg/kg-bw per day.

[12] Estimates of intakes of chloroform from ingestion of drinking water are based on average daily consumption rates of "total tap water" from EHD (1998) and on the maximum concentration of chloroform in drinking water in Canada (i.e., 1224 µg/L, in Alberta) among provincial/territorial data. Total tap water includes water used in the preparation of beverages.

[13] Based on the assumption from Benoit *et al.* (1998) that the combined intake of chloroform from the inhalation and dermal routes during a 10-minute shower is equivalent on an annual average to the intake from ingestion of 2.7 L of cold tap water from the same source. It is assumed that this tap water contains the maximum reported concentration of chloroform in drinking water in Canada (i.e., 1224 µg/L, as in footnote 12).

Table 14: Effect levels in laboratory animals exposed to chloroform by bolus administration (presentation limited to those studies in which the lowest effect levels were reported)

Effect level	**Endpoint**	**Species/sex**	**Protocol**	**Reference**
10 mg/kg-bw per day, LOAEL 3 mg/kg-bw per day, NOEL	Significant increase in liver weight At higher doses: histopathological changes in liver at 34 mg/kg-bw per day; increase in hepatic labelling index at 90 mg/kg-bw per day; degenerative changes in kidney at 34 mg/kg-bw per day; increase in renal labelling index at 180 mg/kg-bw per day	Male F344 rat	Gavage, corn oil, 4 days	Larson *et al.* (1995b)
15 mg/kg-bw per day, LOEL (lowest dose tested)	Fatty cyst, liver; male and female	Beagle dog	Gelatin capsules; 6 days per week for 7.5 years	Heywood *et al.* (1979)
34 mg/kg-bw per day, LOAEL (lowest dose tested)	Increase in hepatic labelling index; histopathological changes (mild centrilobular hepatocyte swelling and pale eosinophilic staining); increase in renal labelling index; necrosis	Male B6C3F1 mouse	Gavage, corn oil, 4 days	Larson *et al.* (1994c)
34 mg/kg-bw per day, LOAEL (lowest dose tested)	Proliferation in olfactory epithelium; nasal lesions At higher doses: Increase in hepatic labelling index and slight centrilobular vacuolar change at 100 mg/kg-bw per day; increase in renal labelling index at 200 mg/kg-bw per day and necrosis	Female F344 rat	Gavage, corn oil, 4 days	Larson *et al.* (1995a)
34 mg/kg-bw per day, LOAEL 10 mg/kg-bw per day, NOEL	Histopathological alterations in liver (pale cytoplasmic eosinophilia of centrilobular hepatocytes and mild vacuolation of centrilobular and midzonal hepatocytes); alanine aminotransferase and sorbitol dehydrogenase significantly increased At higher doses: Hepatocellular proliferation significantly increased at next higher dose, 90 mg/kg-bw per day	Female B6C3F1 mouse	Gavage, corn oil, 3 weeks	Larson *et al.* (1994a)
34 mg/kg-bw per day, LOAEL (lowest dose tested)	Minimal changes in olfactory epithelium	Female F344 rat	Gavage, corn oil, 5 days	Dorman *et al.* (1997)
37 mg/kg-bw per day, LOAEL (lowest dose tested)	Histopathological changes in kidney and liver	Male CD-1 mouse	Gavage, corn oil, 14 consecutive days	Condie *et al.* (1983)

Effect level	**Endpoint**	**Species/sex**	**Protocol**	**Reference**
41 mg/kg-bw per day, LOAEL	Increased liver weight and hepatocellular degeneration in F1 females and increased epididymal weight with ductal epithelial degeneration in males	Swiss CD-1 mouse	Continuous breeding protocol, gavage in corn oil	EHRT (1988)
50 mg/kg-bw per day, LOAEL (lowest dose tested)	Decreased humoral immunity in both sexes and increased relative liver weight in females	Male and female CD-1 mice	Gavage in Emulphor for 14 days	Munson *et al.* (1982)
50 mg/kg-bw per day, LOAEL (lowest dose tested)	Significant dose-related increase in liver weight and hepatic microsomal activity	Female CD-1 mouse	90-day gavage in Emulphor	Munson *et al.* (1982)
50 mg/kg-bw per day, LOEL	Maternal: decreased body weight gain	Female Sprague-Dawley rat	Gavage, corn oil, days 6–15 of gestation	Thompson *et al.* (1974)
50 mg/kg-bw per day, LOEL	Maternal: decreased body weight gain	Female Dutch-belted rabbit	Stomach tube, corn oil, days 6–18 of gestation	Thompson *et al.* (1974)
5 mg/kg-bw per day, LOAEL (lowest dose tested)	Increase in activity of serum alanine aminotransferase and serum sorbitol dehydrogenase; mild hepatocyte hydropic degeneration At higher doses: Increase in hepatocyte labelling index at next higher dose, 110 mg/kg-bw per day	Female B6C3F1 mouse	Gavage, corn oil; 3 weeks, 5 days per week	Melnick *et al.* (1998)
60 mg/kg-bw, LOEL	Significant decrease in liver weight, increase in enzyme activity	Male F344 rat	Gavage, aqueous vehicle, single administration; 24-hour sacrifice	Keegan *et al.* (1998)
60 mg/kg-bw per day, LOAEL	Dose-related increase in both absolute and relative weights of liver, both sexes	Male and female B6C3F1 mice	90-day gavage study, corn oil	Bull *et al.* (1986)
60 mg/kg-bw per day, LOAEL	Dose-related increase in both absolute and relative weights of liver, females only	Male and female B6C3F1 mice	Emulphor vehicle	Bull *et al.* (1986)

Table 15: Effect levels in laboratory animals exposed to chloroform by drinking water (presentation limited to those studies in which the lowest effect levels were reported)

Effect level[1]	**Concentration in drinking water (ppm)**	**Endpoint**	**Species/sex**	**Protocol**	**Reference**
17 mg/kg-bw per day, LOEL	**200**	Foci of regenerating renal proximal tubular epithelium	Male F344 rat	3 weeks	Larson *et al.* (1995b)
6 mg/kg-bw per day, NOEL [intakes reported by authors]	60	At higher levels: At highest dose, mild histopathological changes in liver) (1800 ppm, 106 mg/kg-bw per day)			
38 mg/kg-bw per day, LOAEL	**400**	Histopathological alterations in kidney (Hard and Wolf, 1999; Hard *et al.*, in press)	Male Osborne-Mendel rat	2 years	Jorgenson *et al.* (1985)
19 mg/kg-bw per day, NOAEL [intakes reported by authors]	200				
40 mg/kg-bw per day, LOAEL [intake when exposed to 200 ppm (Health Canada, 1994)]	**200**	Significant increase in labelling index in kidney (medulla only)	Female B6C3F1 mouse	3 weeks	Larson *et al.* (1994a)
12 mg/kg-bw per day, NOEL [intake when exposed to 60 ppm (Health Canada (1994)]	60				
58 mg/kg-bw per day, LOAEL	**1800**	Histopathological alterations in liver; decrease in labelling index in kidney	Male F344 rat	4 days	Larson *et al.* (1995b)
33 mg/kg-bw per day, NOAEL [intakes reported by authors]	400				

[1] Health Canada (1994) conversion factors: For mouse, 1 ppm in water is equivalent to 0.20 mg/kg-bw per day; for rat, 1 ppm in water is equivalent to 0.14 mg/kg-bw per day.

Table 16: Effect levels in laboratory animals exposed to chloroform by inhalation (presentation limited to those studies in which the lowest effect levels were reported)

Effect level	Equivalent effect level (mg/kg-bw per day)[1]	Endpoint	Species	Protocol	Reference
2 ppm, LOAEC 0.3 ppm, NOEC		Proliferation in nasal turbinates	Female B6C3F1 mouse	3 weeks, 7 days per week	Larson *et al.* (1996)
2 ppm, LOAEC (lowest concentration)		Proliferation, nasal passage At higher concentrations: Increase in hepatic labelling index at 300 ppm; minimal renal lesions at 300 ppm; nasal lesions beginning at 10 ppm	Male F344 rat	4 days, 6 hours per day	Templin *et al.* (1996b)
5 ppm, NOEC	40 mg/kg-bw per day	Liver: no change in hepatocyte labelling index; no histopathological changes in liver; no changes in body weight or relative liver weight Kidney: no changes in relative weight, labelling index or histopathology	Male B6C3F1 mouse	3 weeks, 5 days per week, 6 hours per day	Templin *et al.* (1998)
3 or 10 ppm, LOAEC	**20 or 65.2 mg/kg-bw per day**	Increase in relative liver weight at 3 ppm; may have been affected by the perfusion procedure; increase in hepatocyte labelling index at 10 ppm; also mild to moderate vacuolar changes in centrilobular hepatocytes At higher concentrations: Increase in renal labelling index at 300 ppm; no nasal lesions at 288 ppm	Female B6C3F1 mouse	7 days, 6 hours per day	Larson *et al.* (1994b)
5 ppm, LOAEC (lowest concentration)		Ossification of nasal septum	BDF1 mouse (probably both sexes)	2 years, 6 hours per day, 5 days per week	Yamamoto (1996)
10 ppm, LOAEC 3 ppm, NOEC		Cell proliferation, first nasal endoturbinate, histopathological lesions	Male F344 rat	7 days, 6 hours per day	Mery *et al.* (1994)

Effect level	Equivalent effect level (mg/kg-bw per day)[1]	Endpoint	Species	Protocol	Reference
10 ppm, LOAEC 2 ppm, NOEC		Cell proliferation, nasal passages; mild to moderate histopathological lesions At higher concentrations: At 30 ppm, increase in labelling index in kidney; increase in hepatic labelling index and histopathological lesions at 300 ppm	Male F344 rat	3 weeks, 7 days per week, 6 hours per day	Templin *et al.* (1996b)
10 ppm, LOAEC 2 ppm, NOEC		Cell proliferation in nasal passage; minimal to mild histopathological lesions At higher concentrations: Increase in hepatic labelling index and histopathological lesions at 300 ppm; increase in renal labelling index and minimal lesions at 30 ppm	Male F344 rat	6 weeks, 7 days per week, 6 hours per day	Templin *et al.* (1996b)
10 ppm, LOAEC 2 ppm, NOEC		Cell proliferation in nasal passages; mild histopathological lesions At higher concentrations: Increase in hepatic labelling index and histopathological lesions at 300 ppm; increase in renal labelling index at 30 ppm	Male F344 rat	13 weeks, 7 days per week, 6 hours per day	Templin *et al.* (1996b)
10 ppm, LOAEC 2 ppm, NOEC		Proliferation, nasal turbinate; minimal to mild nasal lesions	Female B6C3F1 mouse	4 days, 6 hours per day	Larson *et al.* (1996)
10 ppm, LOAEC 3 ppm, NOEC	**20 mg/kg-bw per day**	Proliferation, first nasal endoturbinate	Female B6C3F1 mouse	7 days, 6 hours per day	Mery *et al.* (1994)
10 ppm, LOAEC (lowest concentration)		Ossification of nasal turbinate	F344 rat (probably both sexes)	2 years, 6 hours per day, 5 days per week	Yamamoto (1996)

Effect level	Equivalent effect level (mg/kg-bw per day)[1]	Endpoint	Species	Protocol	Reference
25 ppm, LOAEC (lowest concentration tested)	**38 mg/kg-bw per day**	Significant increase in relative kidney weight at all doses; cloudy swelling of renal tubular epithelium at all doses; lobular granular degeneration with focal necrosis in liver at all doses	Male rat, strain not specified	6 months, 5 days per week	Torkelson *et al.* (1976)

[1] Based upon conversion factor for chloroform: 1 ppm = 4.9 mg/m^3 (WHO, 1994). 1 mg/m^3 in air is equivalent to intake of 1.33 mg/kg-bw per day for mice and 0.31 mg/kg-bw per day for rats (Health Canada, 1994).

Table 17: Recommended concentrations in media for midpoint and upper-percentile exposure scenarios for PBPK modelling

Medium	**Midpoint estimate**		**Upper-percentile estimate**	
	Conc.	**Developed from**	**Conc.**	**Developed from**
outdoor air (background)	0.14 µg/m^3 (29 ppt)	arithmetic mean from NAPS data (n = 5463) for 1993–1996[1]	0.31 µg/m^3 (63 ppt)	95th percentile from NAPS data (n = 5463) for 1993–1996[1]
outdoor air (commuting)	0.27 µg/m^3 (55 ppt)	arithmetic mean from NAPS data (n = 800) for 4 "road" sites for 1989–1996[1]	0.66 µg/m^3 (135 ppt)	95th percentile from NAPS data (n = 800) for 4 "road" sites for 1989–1996[1]
indoor air (all)	2.28 µg/m^3 (465 ppt)	arithmetic mean from Concord Environmental Corporation (1992) data (n = 754) following lognormal imputation[2]	8.0 µg/m^3 (1630 ppt)	95th percentile from Concord Environmental Corporation (1992) data (n = 754) following lognormal imputation[2]
air in shower compartment	833 µg/m^3 (170 000 ppt)	experimental data assessing the transfer efficiency of chloroform from tap water to shower air, assuming an average concentration[3]	1950 µg/m^3 (398 000 ppt)	experimental data assessing the transfer efficiency of chloroform from tap water to shower, assuming the 95th percentile of the distribution of concentrations[4]
air in bathroom after showering	5 µg/m^3 (1020 ppt)	estimated with the one-compartment model of Blancato and Chiu (1994),[5] assuming a bathroom volume of 13 m^3 and air exchange rate of 2.2 air changes per hour (ACH) from Wilkes *et al.* (1992)[6] and an average concentration of chloroform in tap water	18 µg/m^3 (3670 ppt)	estimated with the one-compartment model of Blancato and Chiu (1994),[5] assuming a bathroom volume of 13 m^3 and air exchange rate of 2.2 ACH from Wilkes *et al.* (1992)[6] and the 95th percentile of the distribution of concentrations of chloroform in tap water
tap water (cold)	47.3 µg/L	arithmetic mean from provincial/territorial data (n = 6607) for 1990–1997[7]	166 µg/L	95th percentile from provincial/territorial data (n = 6607) for 1990–1997[7]
food (all)	0.0035 µg/g	Canadian data for 24 food items[8]	0.0298 µg/g	Canadian and U.S. data for 131 food items[9]

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[1] NAPS data from Dann (1998). Arithmetic mean concentrations were calculated for samples of 24-hour duration. See Health Canada (1999) for further information.

[2] These data are from Concord Environmental Corporation (1992). Twenty-four samples of indoor air were collected using passive sampling devices from 754 homes in nine provinces during 1991 and 1992. At a limit of detection of 3.5 µg/m ^{3}, chloroform was detected in only 10.7% of these indoor air samples. The distribution of concentrations was assumed to be lognormal. Arithmetic mean (i.e., 2.28 µg/m ^{3}) and geometric mean (i.e., 0.72 µg/m ^{3}) concentrations were estimated by lognormal imputation, as described in Health Canada (1999). A 95th-percentile concentration (i.e., 8.0 µg/m ^{3}) was also estimated.

[3] Estimates of the average concentrations of chloroform in the air of a shower compartment during a 10-minute shower were developed in Health Canada (1999) for typical conditions of water temperature (i.e., approximately 40°C) and flow rates (i.e., 5 and 10 L/min), using the arithmetic mean and 95th percentiles of the distribution of concentrations of chloroform in tap water in Canada. A midpoint estimate of the average concentration was developed as follows. At an assumed concentration in water of 50 µg/L (compared with an arithmetic mean concentration of 46.4 µg/L; see Health Canada, 1999) and assuming minimum air exchange between the shower compartment and the adjacent (bathroom) area, estimates of the average concentration of chloroform in the air of the shower compartment during showering ranged from 300 to 1333 µg/m ^{3}. An average concentration of 833 µg/m^3 was selected as the midpoint estimate, based on the assumptions of a water flow rate of 10 L/min and a transfer efficiency of 0.5 (i.e., 50% of the chloroform in the water passing through the shower head is assumed to be volatilized into the air of the shower compartment before the water passes through the shower drain).

[4] An upper-end estimate of the average concentration in the air of a shower compartment during a 10-minute shower was developed in a similar manner. At an assumed concentration in water of 117 µg/L (the 95th percentile of the distribution of concentrations in tap water in Canada; see Health Canada, 1999) and assuming minimum air exchange between the shower compartment and the adjacent (bathroom) area, estimates of the average concentration of chloroform in the air of the shower compartment during showering ranged from 702 to 3120 µg/m ^{3}. An average concentration of 1950 µg/m ^{3} was selected, also based on the assumptions of a water flow rate of 10 L/min and a transfer efficiency of 0.5.

[5] Blancato and Chiu (1994) indicate that the equilibrium relation of the concentration of chloroform in air to the concentration in tap water can be described according to $C_a = (f \times R_w \times C_w) \div (V_b \times R_b)$, where: C_a is the resulting average concentration (mg/m^3) of chloroform in the indoor air; f is the transfer efficiency (i.e., 0.5 assumed; see Health Canada, 1999); R_w is the rate of water use, expressed as L/shower, assuming a flow rate of 10 L/min and a duration of 15 minutes; C_w is the concentration (mg/L) of chloroform in the tap water (i.e., 0.0464 mg/L for the midpoint estimate, and 0.117 mg/L for the upper-percentile estimate); V_b is the volume (m^3) of the bathroom (a volume of 13 m^3 was assumed, based on Wilkes *et al.*, 1992); and R_b is the bathroom ventilation rate (air exchanges/day).

[6] Wilkes *et al.* (1992) estimated a range of air exchange rates: 0.8 ACH (19.2 per day) when the bathroom door is closed; 2.2 ACH (52.8 per day) when the bathroom door is open; and 7.4 ACH (178 per day) when the bathroom door is closed and an exhaust fan is operating. A bathroom ventilation rate of 2.2 ACH was assumed for both the midpoint and upper-percentile exposure scenarios.

[7] Data concerning the distribution of concentrations of chloroform in treated tap water in Canada in the 1990s are summarized in Health Canada (1999).

[8] Ranges of average intakes of chloroform from ingestion of foods for six age groups among the population were developed in Health Canada (1999) using average daily consumption rates (g/day) for 181 food items (EHD, 1998). The minimum intakes in the ranges were based on midpoint estimates of the concentrations of chloroform in 24 specific food items using data originating in Canada only.

[9] The maximum intakes in the ranges were based on midpoint estimates of the concentrations of chloroform in 131 specific food items using data originating in Canada or the United States. For the adult age group, the range of intakes (assuming an average body weight of 70.9 kg) was from 0.084 to 0.71 µg/kg-bw per day. Equivalent intakes in µg/day are 5.96–50.3. The total average daily consumption of the 181 food items by adults is 2353 g (EHD, 1998). Among these, there are two specific food items that are generally prepared using tap water. These are tea (at 317 g/day for adults) and coffee (at 348 g/day for adults). No intake estimates were developed for tea or coffee, as data indicating concentrations of chloroform were not available. The total average daily consumption of 179 food items (i.e., excluding tea and coffee) by adults is (2353 – 665 =) 1688 g. This amount was divided by the minimum (5.96 µg/day) and maximum (50.3 µg/day) of the range of daily intakes to estimate average concentrations of chloroform in the food consumed. The resulting range of average concentrations is 0.0035 µg/g (i.e., midpoint estimate) to 0.0298 µg/g (i.e., upper-percentile estimate).

Table 18: Values of the input parameters[1] representing the midpoint estimates of chloroform concentrations for use in the multimedia exposure scenario

Time (hour)	**QPC (L/min)**	**InhConc (ppm)**	**DermConc (mg/L)**	**DermSurf (cm^2)**	**DWConc (mg/L)**	**DWIngest (L)**	**FdConc (mg/kg)**	**FdIngest (kg)**
0	7.91	4.65e-4	0	0	0.0473	0.16	0.0035	0
0.25	14.55	0.170	0.0473	20 000	0.0473	0	0.0035	0
0.417	14.55	1.02e-3	0	0	0.0473	0	0.0035	0
0.67	14.55	4.65e-4	0	0	0.0473	0	0.0035	0
0.75	14.55	4.65e-4	0	0	0.0473	0.24	0.0035	0.46
1	14.55	5.5e-5	0	0	0.0473	0	0.0035	0
2	14.55	4.65e-4	0	0	0.0473	0	0.0035	0
3	14.55	4.65e-4	0	0	0.0473	0.16	0.0035	0
5	14.55	4.65e-4	0	0	0.0473	0.24	0.0035	0.46
7	14.55	4.65e-4	0	0	0.0473	0.16	0.0035	0
9	14.55	4.65e-4	0	0	0.0473	0	0.0035	0.15
10	14.355	5.5e-5	0	0	0.0473	0	0.0035	0
11	14.55	4.65e-4	0	0	0.0473	0.24	0.0035	0.46
13	7.91	4.65e-4	0	0	0.0473	0.16	0.0035	0
15	7.91	1.02e-3	0.0473	1500	0.0473	0	0.0035	0
15.5	7.91	4.65e-4	0	0	0.0473	0	0.0035	0
16	7.91	4.65e-4	0	0	0.0473	0.16	0.0035	0.15
24	7.91	4.65e-4	0	0	0.0473	0.16	0.0035	0

[1] QPC = alveolar ventilation rate; InhConc = chloroform concentration in air; DermConc = concentration of chloroform in water contacting the skin; DermSurf = skin surface area for dermal absorption; DWConc = concentration of chloroform in drinking water; DWIngest = volume of drinking water ingested; FdConc = concentration of chloroform in food; FdIngest = mass of food ingested.

Table 19: Values of the input parameters[1] representing the 95th percentile of chloroform concentrations for use in the multimedia exposure scenario

Time (hour)	**QPC (L/min)**	**InhConc (ppm)**	**DermConc (mg/L)**	**DermSurf (cm^2)**	**DWConc (mg/L)**	**DWIngest (L)**	**FdConc (mg/kg)**	**FdIngest (kg)**
0	7.91	1.63e-3	0	0	0.166	0.16	0.0298	0
0.25	14.55	0.564	0.166	20 000	0.166	0	0.0298	0
0.417	14.55	3.67e-3	0	0	0.166	0	0.0298	0
0.67	14.55	1.63e-3	0	0	0.166	0	0.0298	0
0.75	14.55	1.63e-3	0	0	0.166	0.24	0.0298	0.46
1	14.55	1.35e-4	0	0	0.166	0	0.0298	0
2	14.55	1.63e-3	0	0	0.166	0	0.0298	0
3	14.55	1.63e-3	0	0	0.166	0.16	0.0298	0
5	14.55	1.63e-3	0	0	0.166	0.24	0.0298	0.46
7	14.55	1.63e-3	0	0	0.166	0.16	0.0298	0
9	14.55	1.63e-3	0	0	0.166	0	0.0298	0.15
10	14.55	1.35e-4	0	0	0.166	0	0.0298	0
11	14.55	1.63e-3	0	0	0.166	0.24	0.0298	0.46
13	7.91	1.63e-3	0	0	0.166	0.16	0.0298	0
15	7.91	3.67e-3	0.166	1500	0.166	0	0.0298	0
15.5	7.91	1.63e-3	0	0	0.166	0	0.0298	0
16	7.91	1.63e-3	0	0	0.166	0.16	0.0298	0.15
24	7.91	1.63e-3	0	0	0.166	0.16	0.0298	0

[1] QPC = alveolar ventilation rate; InhConc = chloroform concentration in air; DermConc = concentration of chloroform in water contacting the skin; DermSurf = skin surface area for dermal absorption; DWConc = concentration of chloroform in drinking water; DWIngest = volume of drinking water ingested; FdConc = concentration of chloroform in food; FdIngest = mass of food ingested.

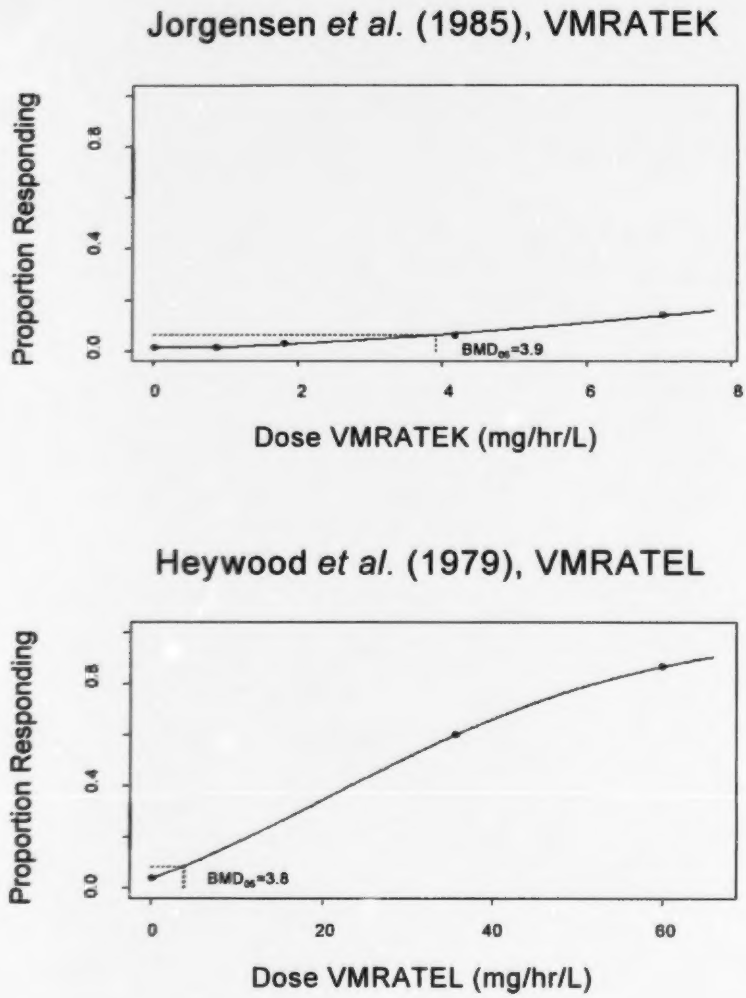

Figure 1: Tumorigenic tissue dose (humans) for combined incidence of renal adenomas and adenocarcinomas in Osborne-Mendel rats (Jorgenson *et al.*, 1985)

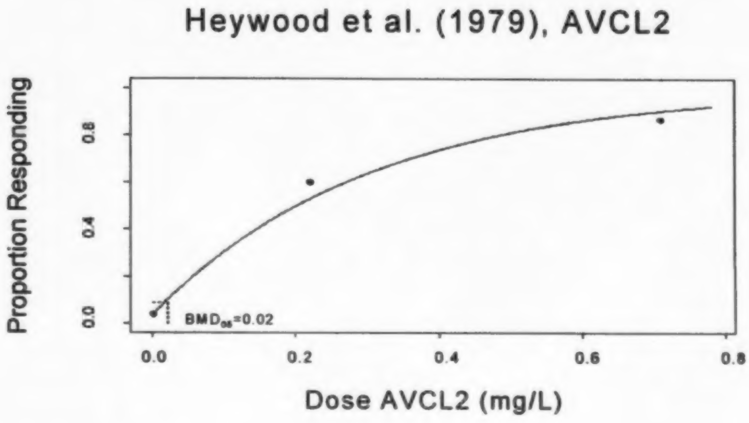

Figure 2: Benchmark tissue dose (humans) for incidence of hepatic fatty cysts in dogs (Heywood *et al.*, 1979)

APPENDIX A: Search strategies employed for identification of relevant data

Environmental assessment

Data relevant to the assessment of the entry, environmental fate and exposure, and environmental effects of chloroform were identified in original literature, review documents, and commercial and government databases and indices, including on-line searches conducted between January and May 1996 of the following databases: ASFA (Aquatic Sciences and Fisheries Abstracts, Cambridge Scientific Abstracts; 1990–1996), BIOSIS (Biosciences Information Services; 1990–1996), CAB (Commonwealth Agriculture Bureaux; 1990–1996), CESARS (Chemical Evaluation Search and Retrieval System, Ontario Ministry of the Environment and Michigan Department of Natural Resources; 1996), CHRIS (Chemical Hazard Release Information System; up to 1985), Current Contents (Institute for Scientific Information; 1993 – January 15, 1996), ELIAS (Environmental Library Integrated Automated System, Environment Canada library; January 1996), Enviroline (R.R. Bowker Publishing Co.; November 1995 – June 1996), Environmental Abstracts (1975 – February 1996), Environmental Bibliography (Environmental Studies Institute, International Academy at Santa Barbara; 1990–1996), GEOREF (Geo Reference Information System, American Geological Institute; 1990–1996), HSDB (Hazardous Substances Data Bank, U.S. National Library of Medicine; 1996), Life Sciences (Cambridge Scientific Abstracts; 1990–1996), NTIS (National Technical Information Service, U.S. Department of Commerce; 1990–1996), Pollution Abstracts (Cambridge Scientific Abstracts, U.S. National Library of Medicine; 1990–1996), POLTOX (Cambridge Scientific Abstracts, U.S. National Library of Medicine; 1990–1995), RTECS (Registry of Toxic Effects of Chemical Substances, U.S. National Institute for Occupational Safety and Health; 1996), Toxline (U.S. National Library of Medicine; 1990–1996), TRI93 (Toxic Chemical Release Inventory, U.S. Environmental Protection Agency, Office of Toxic Substances; 1993), USEPA-ASTER (Assessment Tools for the Evaluation of Risk; U.S. Environmental Protection Agency; up to December 21, 1994); WASTEINFO (Waste Management Information Bureau of the American Energy Agency; 1973 – September 1995) and Water Resources Abstracts (U.S. Geological Survey, U.S. Department of the Interior; 1990–1996).

A survey of Canadian industry was carried out under authority of Section 16 of CEPA, for which companies were required to supply information on uses, releases, environmental concentrations, effects or other data on chloroform available to them if they met the trigger quantity of 1000 kg chloroform per year (Environment Canada, 1997c). Reveal Alert was used to maintain an ongoing record of the current scientific literature pertaining to the potential environmental effects of chloroform. Data obtained after July 1999 were not considered in this assessment.

Human health assessment

To identify data relevant to the estimation of exposure of the general population to chloroform, literature searches were conducted, using the strategy of searching by the name (and major synonyms) and CAS registry number in the following databases: EMBASE (on-line version of Excerpta Medica, Elsevier Science Publishers), Environmental Bibliography (Environmental Studies Institute, Santa Barbara), Enviroline (R.R. Bowker Publishing Co.), NTIS (National Technical Information Service, U.S. Department of Commerce), Pollution Abstracts (Cambridge Scientific Abstracts), Waternet (American Water Works Association) and Water Resources Abstracts (Cambridge Scientific Abstracts).

To identify the toxicological data relevant to the assessment of chloroform, literature searches were conducted, using the strategy of searching by the name or CAS registry number in the following databases: CCRIS (Chemical Carcinogenesis Research Information System, U.S. National Cancer Institute), CESARS (Chemical Evaluation Search and Retrieval System, Ontario Ministry of the Environment and Michigan Department of Natural Resources), DART (Developmental and Reproductive Toxicology, U.S. Environmental Protection Agency and U.S. National Institute of Environmental Health Sciences), EMIC (Environmental Mutagen Information Center, Human Genome and Toxicology Program, Oak Ridge National Laboratory), GENE-TOX (Genetic Toxicology, U.S. Environmental Protection Agency), HSDB (Hazardous Substances Data Bank, U.S. National Library of Medicine), IRIS (Integrated Risk Information System, U.S. Environmental Protection Agency), RTECS (Registry of Toxic Effects of Chemical Substances, U.S. National Institute of Occupational Safety and Health) and Toxline Plus (U.S. National Library of Medicine).

Subsequent to these searches, data relevant to both environmental exposure and health effects were identified through an SDI (Selective Dissemination of Information) profile. Data obtained after October 1999 were not considered in this assessment.